DETERMINATION OF PROCESSING CONDITIONS FOR INDUSTRIAL MANUFACTURE OF PRE-DENATURED WPC

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ABSTRACT

The purpose of this study was to establish processing conditions for manufacturing denatured whey protein concentrate (WPC) with the ability to form self-supporting gels upon addition of water with minimal or no heating. Such product could be used as thickening and gelling agents in various food applications.

In the preliminary studies, fresh whey and ultrafiltrate (UF) retentate solutions were heated and analysed using polyacrylamide gel electrophoresis (PAGE). The results showed that heating 1% protein solution of retentate (pH 7.0) at 80°C for 20 min formed the desired "soluble" aggregates. Those aggregates were predominantly formed at lower protein concentration compared to that at higher protein concentration. Much larger aggregates were formed when acid whey was heated under similar conditions.

The same heating conditions (1% retentate solution, pH 7.0, 80°C for 20 min) were used in two different pilot plant trials (Massey University and Anchor Products) to produce denatured WPC powders. The denatured WPC powders were capable of forming viscous solutions or gels at ~ 10% protein concentration upon re-hydration and addition of GDL, $CaCl_2$ or NaCl at ambient temperatures. By contrast, the unheated WPC solutions did not gel under these conditions. The viscosity or gel strength of the denatured WPC solutions increased with protein concentration, incubation time and temperature in the presence of additives.

The heat-denatured WPC powders produced in the pilot plants had desirable functional properties. The high viscosity and the ability to form a gel upon addition of GDL or salts at 5-40°C would enhance their application in food systems, such as in comminuted meat, pressed ham/bacon, mayonnaise and yoghurt products.

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TABLE OF CONTENTS

CHAPTER 1
INTRODUCTION

Whey proteins have been widely used to enhance the nutritional value and various functional properties of many food products, for example, infant formula, health foods and drinks, frozen foods. Whey protein concentrate (WPC) is a major whey protein product in dairy industry and has attracted considerable attention as a potential food ingredient during the last two decades. The New Zealand WPC products are sold at premium prices in the international markets, largely because of their highly desired functional properties, such as gelation, emulsification and foaming. However, the functional applications of WPC products are still rather limited compared to the market expectations. Being aware of competitors attempting to replace the present products, much research is needed to enhance the functionality of WPC products using innovative technologies to produce new WPC products with superior functionality.

One of the applications of WPC in food systems is where minimal or no heat treatment is required. In these applications, WPC is pre-denatured or pre-gelatinised and upon re-hydration under appropriate conditions, forms a gel. Researchers in this area have largely been using extrusion technologies, where a combination of heat and shear results in a denaturation and aggregation of whey proteins. Generally a standard WPC powder is used with addition of water to a certain concentration (30 to 39% of total solids) suitable for extrusion conditions. The extrudate is then dried and milled into a powder form. The powder can be used as a stabiliser or thickener in products such as ice cream, yoghurt and other dairy desserts. However, commercial production of WPC using the above approach has a few associated problems. Firstly, a WPC powder is used for the extrusion process, which means that the WPC powder is being further processed by extrusion and milling to get a "secondary" powder - the final product. Secondly, the extrusion process does not usually provide strict control of the rates of denaturation and aggregation that is critical in the manufacture of products with desired functionality. The final denatured or gelatinised product is usually undesirable for some intended applications because of inconsistent particle sizes in the powder. Thirdly, the cost of extrusion is very high and does not justify production in many cases. Therefore, understanding of how to control the degree of thermal

denaturation and aggregation of whey proteins is the key point in developing novel processes for WPC products with desired functionality.

Research on the thermal properties of WPC have showed that the nature of aggregates formed during heat-induced denaturation vary strongly with experimental heating conditions (McSwiney *et al.*, 1994a,b; Gezimati, *et al.*, 1997; Havea, 1998; Havea *et al.*, 1998). For example, large aggregates are formed when whey proteins are heated at low pH, high protein concentration, high ionic strength or at fast heating (high-temperature) rates. More detailed information on the effects of environmental conditions on the size of the aggregates formed is desirable to permit optimisation of heat treatments that ensure the formation of specially sized and shaped aggregates, which give the desired functional characteristics to the WPC products.

Recent studies by Professor Harjinder Singh's research group at Massey University (unpublished) have indicated that the size and type of protein aggregates formed in heated solutions of whey proteins could be manipulated by controlling the ionic strength, heat treatment, pH and protein concentration. The results could be applied to develop a novel process for producing WPC products containing pre-aggregated whey proteins, capable of formation of gels at ambient temperature.

In recent years, a technique, namely cold-set gelation, has been introduced to produce whey protein ingredients that are capable of thickening solutions or creating gels at ambient and even refrigeration temperatures (Barbut & Foegding, 1993; Kawamura *et al.*, 1993; Hongsprabhas & Barbut, 1996; Ju & Kilara, 1998a). In such cold-set gelation of WPC or WPI, preheating is an essential step required for denaturing the whey proteins, followed by incubation with additions of salts, acidulants or enzyme. The preheating is considered to be responsible for providing a significant degree of protein denaturation and aggregation, which have the effect of producing high viscosity and good gelation properties of the final WPC or WPI products. The gel properties depend on preheating conditions as well as factors affecting denaturation and aggregation of whey proteins, and subsequent gel induction methods. However, most studies on the cold-set gelation focused only on WPI and limited to a laboratory level. Knowledge on the effects of preheating on the formation and development of aggregates as well as preheating and incubation conditions on the properties of cold-set gels is still limited. There is no information available on the

production of heat-denatured WPC products and factors affecting cold-set gelation of denatured whey proteins in WPC system.

The purpose of this study was to determine the appropriate processing conditions (protein concentration, heating temperature and time, pH and ionic strength) for commercial production of a heat-denatured WPC product, capable of gel formation upon re-hydration with minimal or no further heat treatment. The processing conditions, determined in bench-scale fundamental work, were tested in pilot plant trials to produce concept of denatured WPC products. In addition, the functional properties of heat-denatured WPC powders produced were investigated.

CHAPTER 2
LITERATURE REVIEW

2.1. Introduction

Derived from whey, whey protein concentrate (WPC) and whey protein isolate (WPI) are major whey protein products widely used as food additives in meat, beverage, dairy product, baked food and infant formula because of their superior nutritional values and highly desired functional properties.

One of the most interesting functional properties of WPC is the ability to form heat-induced gels, capable of holding large amounts of water and other nonprotein components after being denatured (Mangino, 1992). It has been suggested that heat-induced gelation of whey proteins involves a series of stages including denaturation, aggregation and strand formation. The gelation, therefore, is affected by the extent of protein denaturation and formation of "soluble" aggregates being governed by the chemical and physical properties of the main protein constituents: β-lactoglobulin (β-Lg), α-lactalbumin (α-La), bovine serum albumin (BSA) and immunoglobulins (Ig).

The major whey proteins have different amino acid compositions, structures and properties, and the functional behaviour of an individual protein in denaturation, aggregation and gelation can be altered by the presence of the others and the environmental conditions. These have lead to extensive studies of individual whey proteins and their denaturation and aggregation during heating as well as the effects of factors such as protein concentration, pH, heating temperature and time, ionic strength and the presence of other ingredients (de Wit & Klarenbeek, 1984; Matsudomi *et al.*, 1992, 1993; McSwiney, *et al.*, 1994a,b; Nielsen *et al.*, 1996; Gezimati *et al.*, 1996, 1997; Havea, 1998; Havea *et al.*, 1998; Hoffmann & van Mil, 1999). Better understanding of individual whey proteins and their behaviour under various heating conditions is important for controlling of industrial processing of WPC to develop new products with predictable or tailor-made functional properties.

Cold-set gelation is a new concept recently developed to produce whey protein ingredients that are capable of thickening solutions or creating gels at ambient or even refrigeration temperature upon addition of salts, acid or enzyme (Barbut & Foegding, 1993; Kawamura *et al.*, 1993; McClements & Keogh, 1995; Hongsprabhas & Barbut, 1997a,b; Ju & Kilara, 1998a,b). Such cold-gelling ability has potential applications in

the food industry. A commercial whey protein product for the purpose of cold-set gelation has been manufactured (Thomson, 1995), which can be used in various foods, such as surimi, pressed ham, dressing, spreads and bakery products. However, knowledge of the effects of preheating on the formation of aggregates and incubation conditions on the properties of cold-set gels is still limited.

This review provides a brief overview of general characteristics of whey proteins and industrial manufacture of WPC, a discussion of thermal denaturation and aggregation behaviour of the major whey proteins, and the effects of the environmental conditions on these behaviours during heat-induced and cold-set gelation.

2.2. Whey and whey proteins

Whey, a by-product of cheese and casein manufacture, can be defined as the liquid remaining after removal of casein from milk. Whey was traditionally regarded as a waste product and was generally disposed of as effluent or as animal feed. However, a worldwide shortage of high-quality animal proteins, as well as high cost of whey treatment, has increased the interest in whey recovery and processing in dairy industry.

There are generally two types of whey, depending on the means used for separating casein. Sweet whey (rennet and cheese whey) is produced after casein is separated from milk by addition of rennet, while acid whey is produced after removal of casein from milk by direct addition of mineral acid or by *in situ* production of lactic acid from starter bacteria.

2.2.1. Composition of whey

Whey comprises 80-90% of the total volume of milk entering the process and contains about 50% of the nutrients in the original milk: soluble protein, lactose, vitamins and minerals. The composition varies depending on the milk source, the processing methods used and the cheese or casein type (Mulvihill & Donovan, 1987). Typical composition of sweet and acid whey is shown in Table 2.1. Sweet whey has a higher pH, total solids, protein and lactose concentrations but lower concentration of calcium and potassium than acid whey.

Table 2.1. Typical composition of acid and sweet whey

Component	Composition (g/l)	
	Acid whey	Sweet whey
Total solids	63 - 64	66 - 67
Total protein	6.1 - 6.2	6.5 - 6.6
Lactose	44.3 - 46.9	52.3 - 52.4
Minerals	7.5 - 7.9	5.0 - 5.2
Calcium	1.4 - 1.6	0.4 - 0.5
Potassium	2.0	0.5 - 1.0
Sodium	0.5	0.5
pH	4.6 - 4.7	5.9 - 6.4

(Adapted from Mulvihill & Donovan, 1987)

2.2.2. Whey proteins

Normal bovine milk contains 30-35g/L total proteins. Whey proteins, with approximately 20% of the original milk proteins, can be defined as those proteins remaining soluble at pH 4.6 at 20°C after casein removal from whole or skim milk (Mulvihill & Donovan, 1987). The major whey proteins are β-lactoglobulin (β-Lg), α-lactalbumin (α-La), bovine serum albumin (BSA), immunoglobulins (Ig) and proteose peptones (PP). Except the PP, whey proteins are globular to ellipsoid in structure and are relatively soluble and heat labile. Forces involved in maintaining the structure of whey proteins include hydrophobic interactions, disulphide bonds, hydrogen bonds and electrostatic interactions (Kinsella & Whitehead, 1989). Some notable characteristics of major whey proteins are listed in Table 2.2.

2.2.2.1. β-Lactoglobulin (β-Lg)

First crystallised in 1934, β-Lg is the dominant whey protein and comprises more than 50% of total whey proteins in bovine milk. The monomeric unit of β-Lg, molecular weight of 18,400 Da, possesses 162 amino acid residues with one free thiol group (Cys^{121}) and two disulphide bonds (Cys^{66} - Cys^{160} and Cys^{106} - Cys^{119}) (Papiz *et al.*, 1986). Among seven generic variants identified, most common variants occurring

at almost equal frequency are β-Lg A and β-Lg B, which only differ at positions 63 and 118, where an Asp and a Val in β-Lg A are substituted by a Gly and an Ala in β-Lg B (Eigel *et al.*, 1984).

Table 2.2. Physicochemical characteristics of whey proteins in bovine milk

Whey protein	Concentration in milk (g/kg)	MW (Da)	Isoelectric point	Disulphide bonds
β-Lactoglobulin	3.3	18,363	5.13	2
α-Lactalbumin	1.2	14,147	4.2-4.5	4
Bovine serum albumin	0.4	66,267	4.7-4.9	17
Immunoglobulin	0.7	$(1.5\text{-}10) \times 10^5$	5.5-8.3	21
PP & other minor whey proteins	0.8	$(4.1\text{-}40.8) \times 10^3$	-	0

(Adapted from Walstra & Jenness, 1984 ; Mulvihill & Donovan, 1987)

The native conformation of β-Lg is sensitive to heat and pH. At room temperature, β-Lg usually exists as a stable dimer between pH 5.5 and 7.5 because of electrostatic interactions between Asp^{130} and Glu^{134} of one monomer with corresponding lysyl residues of another monomer (McKenzine, 1971; Creamer *et al.*, 1983). However, the dimer dissociates into monomers at pH below 3.5 due to strong electrostatic repulsion or at elevated temperature. The dissociation is enhanced by a low protein concentration, a low ionic strength, further decrease in pH, and increasing temperature. At pH between 5.2 and 3.5, β-Lg undergoes reversible tetramerization to form octamer, especially at a temperature below 20°C. Conformational changes in β-Lg are induced at pH values exceeding 8.0, where irreversible time-dependent aggregation occurs due to intermolecular disulphide bonds (McKenzine, 1971; Mulvihill & Donovan, 1987).

2.2.2.2. α-Lactabumin (α-La)

α-La is the second major globular protein present in the whey. It accounts for approximately 20% of whey proteins. The primary structure consists of 123 amino

acid residues (molecular weight of 14,200 Da) with four disulphide bonds located between 6 and 120, 28 and 111, 61 and 77, 73 and 91 (Eigel *et al.*, 1984). The protein forms dimers and trimers at pH values below its isoelectric point (pH 4.2). Above pH 9.0 and below pH 4.0, α-La undergoes conformational changes without causing irreversible aggregation, while conformation is stable between pH 5.4 and pH 9.0 (Lyster, 1972). Upon heating, reversible conformational changes in α-La can be detected at pH near neutrality, but no precipitation occurs.

2.2.2.3. Bovine serum albumin (BSA)

As a transport protein for insoluble fatty acids in the blood circulatory system, BSA has the longest single polypeptide chain of all the whey proteins, consisting of 582 residues and has a molecular weight of 66,000 Da. It has one free thiol group at position 34 and 17 disulphide bonds (Eigel *et al.*, 1984; de Wit, 1989).

2.2.2.4. Immunoglobulin (Ig)

Bovine Ig, accounts for ~ 10% of whey protein, is a set of glycoproteins that possess antibody activity. There are four distinct classes: IgM, IgA, IgE and IgG (IgG_1 & IgG_2) with molecular weights varying 150 to 900 KDa. All classes exist as either polymers or monomers of a basic four-chain unit composed of two identical light (22,000 Da) and two identical heavy (50,000-70,000 Da) polypeptide chains linked covalently by disulphide bridges (Eigel *et al.*, 1984).

2.3. Whey protein concentrate

Whey protein concentrate (WPC) containing 35-85% of proteins is a major whey protein product developed to date in the dairy industry. The quality of source whey is very important for high quality WPC with consistent composition and functional properties. It is particularly necessary to minimise the psychrotropic and thermoduric microorganisms during the processing because proteolytic activities caused by organisms can influence the functional properties of WPC and excessive numbers of thermoduric bacteria can result in acid production during the manufacture of WPC (Hobman, 1992).

2.3.1. Manufacture of WPC

Several processes for producing WPC have been available to the industry for years: heat coagulation, gel filtration, electrodialysis, polyphosphate precipitation, ion exchange and ultrafiltration. Among these, ultrafiltration (UF) and diafiltration (DF) are the preferred technology for manufacturing WPC in modern industries. A simplified schematic flow diagram for production of WPC from both acid and sweet whey is shown in Figure 2.1. The processing can be divided into three stages: whey pretreatment, UF and DF, concentration and drying.

2.3.1.1. Whey pretreatment

This procedure is carried out to improve flux and reduce fouling during UF or DF, and to manipulate the chemical composition or functional properties of WPC. There is a range of pretreatment processes used in the dairy industry, generally including clarification, preheating and pasteurisation and preconcentration.

Clarification is to remove those soluble or insoluble components that have the potential to cause fouling of the membranes and reduce flux during UF, such as residual fine particles of casein or cheese curd, fat, the bulk of bacterial starter cells, microorganisms and mineral precipitates. Centrifugal clarifier and rotary screen filter are widely used commercially (Hobman, 1992).

Preheating the whey to a temperature above that to be used during UF (e.g. typically 50°C) and holding for a period time is generally considered to be effective in reducing fouling of the membranes because it tends to stabilise the calcium phosphate component of whey. Cheese whey is commonly pasteurised at 72°C for 15 s and stored at low temperature (e.g. < 6°C) prior to further processing. In comparison, acid whey is generally not pasteurized because such a heat treatment at the natural pH of the whey (e.g. pH 4.6) can result in denaturation of the protein. Moreover, acid whey is stored at a temperature exceeding 52°C, which is sufficiently inhibitory to the growth of microorganisms to avoid the need for pasteurization (Hobman, 1992).

Preconcentration is applied to reduce whey transportation costs, storage volumes and energy consumption. It is also possible to improve fat separation, increase total solids of retentate and permeate, and to reduce the quantity of water to be removed during evaporation and drying (Nielsen, 1988).

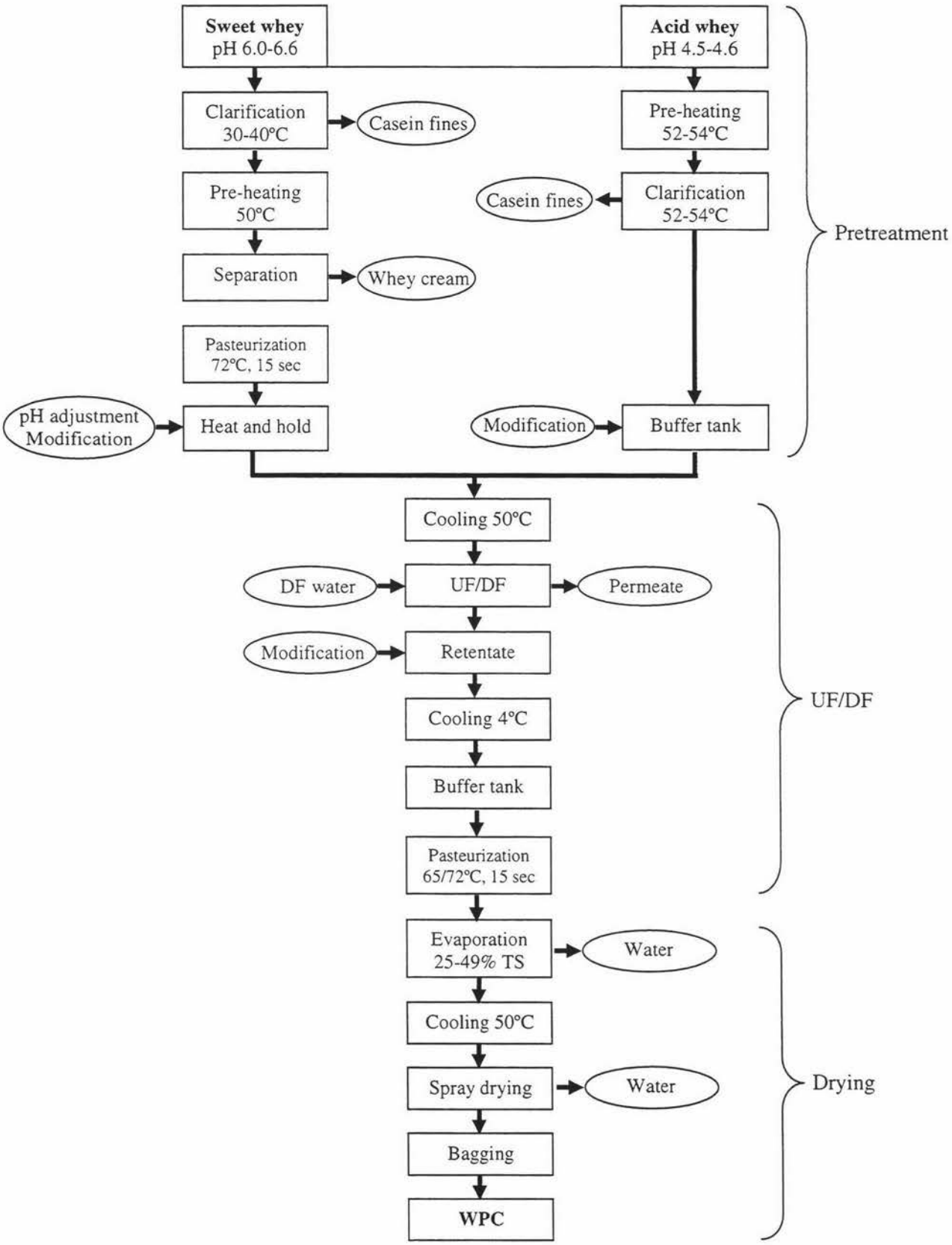


Figure 2.1. Simplified schematic flow diagram for the production of WPC (from Nielsen, 1988).

2.3.1.2. UF and DF

UF is a pressure-driven filtration process in which porous membranes are used to separate the components of a solid-liquid mixture on the basis of size and shape, and, in some instances, charge (Hobman, 1992). The pore dimensions are typically in the range of 1-100 nm nominal diameter. When a pressure gradient is applied across the membrane, the liquid is forced to flow through the pores to the low-pressure side, transporting with it any components that are smaller than the size of the membrane pores.

UF enables the whey proteins to be separated from the lactose, minerals and other water-soluble, low-molecular-weight species. The retentate, therefore, obtained from UF contains higher molecular weight materials (proteins and fat), water, and small proportions of low molecular weight materials whereas the permeate consists of water, lactose, vitamins and amino acids (Hobman, 1992). The optimum temperature used in UF process of whey is generally considered to be 50°C. At such temperature, it is possible to achieve acceptable fluxes and avoid the problems of microorganisms and thermal denaturation of proteins.

Membrane selection is of major importance in UF process. It should ensure to achieve the desired separation and acceptable operating cost. Tubular membranes, with a wide range of porosity and separability in molecular mass of 500 to 200,000, are most popular in modern UF applications because of their simplicity and versatility. Another critical factor for UF process is the shear stress at the membrane surface. The chosen shear stress must be of a sufficiently high value to avoid the formation of a gel layer at the membrane and enable attainment and maintenance of high flux (Hobman, 1992).

DF is employed to produce WPC with protein contents over 65%. It is a process by which the retentate is diluted with water and further ultrafiltered, which allows further removal of low molecular weight material from retentate. The quality of water used in DF is of particular important to avoid membrane fouling. Demineralized or softened water or water from other suitable sources (e.g. evaporator condensate and reverse osmosis permeate) is commonly used (Hobman, 1992). The number of DF stages and the volume of water used are dependent on the design and operation of the UF plant, as well as the WPC specification.

2.3.1.3. Concentration and drying

The retentate obtained from UF plant need to be cooled (~ 4°C) before further process. Pasteurisation of the retentate using a heat treatment of 66-72°C for 15 s may be necessary to reduce the number of bacteria as bacteria in whey can be concentrated up to 130 times during UF for an 80% protein product (Nielsen, 1988).

Before drying, the retentate can be concentrated by a high-vacuum, low-boiling temperature (e.g. 50°C) failing-film evaporator to minimise the cost of water removal and improve the physical properties of the powder. The products containing 35-80% protein are able to be concentrated to 25-44% total solids, while avoiding thermal denaturation of the protein (Nielsen, 1988).

Drying of retentate is usually performed using a spray drier fitted with nozzle atomisation. Typically, the inlet and outlet air temperatures used are 160-180°C and less than 80°C, respectively (Hobman, 1992).

2.3.2. Composition of WPC

The composition of WPC, which reflects the source of the whey, can also be largely influenced by the processing history. Depending on the degree of UF (and on the steps of DF applied), WPC can be produced with enormously varying compositions. Table 2.3 shows the average composition of eight commercial WPCs investigated (Morr & Foegeding, 1990). The functionality of WPC in food system will be largely different owing to the differences in protein contents. The WPC containing 35-55% protein are used for animal feed manufacture while WPC that contain over 70% protein are used extensively as functional and nutritional ingredients in medical, pharmaceutical and human food products, such as infant formula, health food and drinks, and frozen foods (Morr & Ha, 1993).

The contents of other components also have effect on WPC functionality. Lipid content of WPC tends to increase as the protein content increases. Removal of residual lipid from whey has been shown to increase the UF flux and to improve WPC functionality. The concentration of lactose in WPC generally decreases as the concentration of protein increases. Values for the lactose content of commercial available WPC range from 0.1 to 46%. Lactose can increase the heat stability of proteins and react with proteins via non-enzymeic browning, producing less nutritious

and lower functional products (Hobmann, 1992). Different methods used for processing WPC cause different content of minerals. The ash contents for commercially available WPC produced by UF, electrodialysis and metaphosphate complex formation ranged from 0.98 to 12.18%. Among these minerals, calcium has received most attention regarding its effect on functionality of WPC. Commercially available WPCs, both acid and sweet, contain calcium at a level where it weakens the gel strength and thus, the gel strength generally increases with a reduction of calcium (Mangino, 1992).

Table 2.3. Chemical composition of commercial WPCs

Component	WPC	
	Range	Mean ± SD
Moisture	4.14 – 6.01	5.31 ± 0.66
Protein	72.0 – 76.6	73.8 ± 1.64
Lactose	2.13 – 5.75	3.92± 1.20
Total lipids	3.30 – 7.38	5.00 ± 1.27
Phospholipids	0.80 – 1.54	1.28 ± 0.23
Ash	2.52 – 6.04	4.28 ± 1.29
Sodium	0.15 – 1.71	1.04 ± 0.65
Potassium	0.07 – 0.46	0.25 ± 0.17
Calcium	0.23 – 1.05	0.46 ± 0.27
Magnesium	0.02 – 0.40	0.09 ± 0.12
Phosphorus	0.20 – 1.30	0.44 ± 0.35

(Adapted from Morr & Foegeding, 1990)

2.4. Denaturation and aggregation of whey proteins

The globular structure of whey protein is mainly maintained by covalent disulphide linkage and non-covalent interactions such as hydrogen bonding, van der Waals force, hydrophobic and electrostatic interactions (Mulvihill & Donovan, 1987). Any major alteration in secondary, tertiary and quaternary structures of the protein

without hydrolysis of primary covalent bonds can be defined as protein denaturation (de Wit, 1981; Mulvihill & Donovan, 1987). Denaturation of whey proteins is generally assumed to be a process consisting of at least two steps (Mulvihill & Donovan, 1987; de Wit, 1990): initially a partial unfolding of the native protein and a subsequent aggregation.

Once physical or chemical agents rupture the forces stabilising the native structure, the native protein collapses to a random coil structure. It exposes reactive side chain groups originally buried within the native structure. The second step involves aggregation of unfolded protein molecules through thiol-disulphide interchange reactions and hydrophobic interactions (de Wit, 1984; Mulvihill & Donovan, 1987; Kella & Kinsella, 1988).

Denaturation of whey proteins can be caused by a variety of physical (heating, hydrostatic pressure, shear) and chemical (pH, organic solvent and solutes, detergents, chaotropic salts) denaturing agents. Among these, heating is the most important denaturant.

Various techniques, including polyacrylamide gel electrophoresis (Dannenberg & Kessler, 1988; McSwiney *et al.*, 1994b; Havea *et al*, 1998), differential scanning calorimetry (Qi *et al.*, 1995: Boye *et al*, 1997), solubility at pH 4.6 (Donovan & Mulvihill, 1987; Law & Leaver, 1997), high-performance liquid chromatography (Matsudomi *et al.*, 1992, 1994; Havea, 1998), optical rotatory dispersion and circular dichroism (Iametti *et al.*, 1995), light scattering (Hoffmann *et al.*, 1996) have been applied to determine the rate of denaturation of whey proteins.

2.4.1. Thermal denaturation behaviour of individual whey proteins

Individual whey proteins exhibit different thermal denaturation behaviours in different system. For example, the resistance of whey proteins to heat denaturation in milk (Dannenberg & Kessler, 1988; Singh & Creamer, 1991; Oldfield *et al.*, 1998) and rennet whey (Donovan & Mulvihill, 1987) follows the order: α-La > β-Lg > BSA > Ig. However, de Wit & Klarenbeek (1984) reported a different resistant order of whey proteins in individual protein system (8-10% of protein concentration at pH 6.0 in 0.7 M phosphate buffer): β-Lg > Ig > BSA > α-Lg. Besides, the behaviour is complicated by the effects of heating conditions, pH, protein concentration, ionic

strength (especially calcium) and the presence of chelating agents such as citrate and phosphate.

Different genetic variants of β-Lg differ in heat stability. There is no agreement on which one between β-Lg A and β-Lg B is more heat stable. A number of researchers have shown that β-Lg B is denatured more rapidly than β-Lg A in skim milk (Dannenberg & Kessler, 1988), cheese whey (Hillier & Lyster, 1979), or solution of isolated proteins (Mckenzie, 1971). However, Boye *et al.* (1997) using DSC, showed β-LgB was more stable to heat treatment than β-Lg A, which confirmed the results reported by McSwiney *et al.* (1994b), who used solution containing 10% (w/v) protein. Nielsen *et al.* (1996) indicated that the irreversible denaturation of β-Lg was concentration dependent. This finding may provide an explanation of these contradictory results. In the study by Nielsen *et al.* (1996), it was observed that at concentration of 1.25 or 2.5% (w/v), β-Lg B was more sensitive to heat treatment at 75°C than β-Lg A. On the other hand, the result obtained at protein concentrations of 10% or 15%, showed that β-Lg A denatured faster than β-Lg B. Qi *et al.* (1995) also reported in an earlier study that both the scanning rate and protein concentration have an effect on the denaturation temperature of β-Lg, which further confirmed the findings of Nielsen *et al.* (1996). The rate of denaturation of variants A and B was also dependent on the pH and composition of the medium, as well as different heating methods and analytical techniques (McSwiney *et al.*, 1994b).

Of the whey proteins, α-La has the lowest denaturation temperature at 62°C, followed by BSA at 64°C, Ig at 72°C and β-Lg at 78°C (Table 2.4). β-Lg denatures more quickly than α-La at 85°C and at other temperatures in heated milk and whey systems, and the overall effect of heating is greater on β-Lg than on α-La because α-La can renature upon cooling (Rüegg *et al.*, 1977; Donovan & Mulvihill, 1987). However, de Wit & Klarenbeek (1984) found that, although purified α-La renatured upon cooling, the α-La present in heated WPC did not renature upon cooling. This was supported by Hollar *et al.* (1995), who reported that α-La denatured more extensively than β-Lg at 66°C and 77°C in a WPC solution.

The thermo-sensitivity of BSA, which has been reported to have denaturation temperature, of 62.2°C, 64°C or 72-74°C depending on pH (de Wit & Klarenbeek,

1984). BSA has an irreversible thermal transition with the free thiol group being responsible.

Table 2.4. Thermal denaturation temperatures and enthalpies of whey proteins

Whey protein	Td (°C) [a]	Ttr (°C) [b]	ΔH (kl/mol) [c]
β-Lactoglobulin	78	83	311
α-Lactalbumin	62	68	253
Bovine serum albumin	64	70	803
Immunoglobulins	72	89	500

[a] Initial denaturation temperature; [b] Temperature at DSC peak maximum;
[c] Enthalpy of denaturation.

(Adapted from de Wit, 1984)

2.4.2. Heat-induced aggregation of whey proteins

The aggregates of heat-denatured protein molecules can be formed through intermolecular hydrophobic interactions, disulphide bonds, hydrogen bonds, or electrostatic interactions (Kinsella & Whitehead, 1989). Hydrophobic interactions and disulphide bonds are considered the strongest contributors to developing stable aggregates and gel networks in whey proteins. The extent of their relative contribution to the aggregation and gelation process is unclear and depends on experimental conditions, such as pH, heating temperature, protein concentration and ionic strength (Mulvihill & Donovan, 1987; McSwiney *et al.*, 1994a; Hoffmann *et al.*, 1997).

2.4.2.1. Aggregation of individual whey proteins

McKenzie (1971) suggested that denaturation and aggregation of β-Lg at neutral pH involved several steps with a number of intermediate species and that the reactions included dissociation of the native dimer to monomer, monomer conformational changes, disulphide interchange to form aggregates, oxidation of thiols to disulphides and noncovalent aggregation. This hypothesis was supported by the more recent results of Qi *et al.* (1995).

Heat-induced aggregation of β-Lg was investigated as a function of pH, heating temperature, protein concentration, ionic strength by various techniques including measurements of reaction kinetics, SDS-PAGE, DSC, light scattering, high-performance size-exclusion chromatography (McSwiney *et al.*, 1994a,b; Elofsson *et al.*, 1996; Nielsen *et al.*, 1996; Hoffmann *et al.*, 1996; Prabakaran & Damodaran, 1997; Havea, 1998; Havea *et al.*, 1998; Manderson *et al.*, 1998; Verheul *et al.*, 1998; Havea *et al.*, 2000; Hoffmann *et al.*, 1999).

Prabakaran & Damodaran (1997) reported that changes in the secondary structure of β-Lg occurred at 63°C. Results of Hoffmann *et al.* (1996) have also indicated that the initial rate of heat-induced aggregation of β-Lg was significant only above 61.5°C. The reactive monomer initially forms a reactive dimer via thiol-disulphide exchange reaction with another reactive monomer. When the dimer concentration reaches a critical level, continued thiol-disulphide interchange reactions lead to the formation of higher molecular weight polymers (Prabakaran & Damodaran, 1997). The dimers could be important intermediates in the further aggregation of β-Lg (Manderson *et al.*, 1998). The formation of higher molecular weight aggregates (trimers, tetramers, etc.) probably involves interactions between the exposed thiol group and disulphide bonds of two dimers or between those of a dimer and another reactive monomer (Havea, 1998; Manderson *et al.*, 1998).

There was a marked difference between the aggregation behaviour of the two variants of β-Lg. Below a protein concentration of 5%, the aggregation were faster for β-Lg B than for β-Lg A but above 5%, β-Lg A appeared to be more sensitive to thermal aggregation (Nielsen *et al.* 1996). Manderson *et al.* (1998) later confirmed that the proportion of large aggregates formed from 3-4 mg/ml β-Lg at pH 6.7 was: β-Lg B > β-Lg A. In an earlier study, Parris *et al.* (1993) showed β-Lg B formed soluble smaller sized aggregates, whereas β-Lg A formed mainly large insoluble aggregates in sweet whey.

It is generally believed that α-La does not aggregate when heated alone under mild conditions (75°C or 80°C, pH 6.7-7.0) (de Wit & Klarenbeek, 1984; Matsudomi *et al.*, 1992; Hines & Foegeding, 1993; Dalgleish *et al.*, 1997; Gezimati *et al.*, 1997; Havea, 1998; Havea *et al.*, 1998; Havea *et al.*, 2000). The lack of aggregate formation is largely explained by the lack of a free thiol group in the molecular structure (Eigel

et al., 1984). The presence of other proteins, especially those containing free sulfhydryl groups, such as β-Lg or BSA, allows interactions to occur between α-La and the added protein (Gezimati *et al.*, 1997; Havea *et al.*, 2000). When α-La is heated under more severe conditions (100°C for 10-30 min), disulphide-linked polymers as well as modified monomers are formed (Chaplin & Lyster, 1986). The later are probably in the "molten globule state" (Kuwajima, 1989; Hirose, 1993).

BSA is one of the most heat sensitive among whey proteins under a range of heating conditions at near neutral pH (de Wit & Klarenbeek, 1984). Matsudomi *et al.* (1993) had previously reported that BSA treated with N-ethylmaleimide (NEM), a thiol-blocking reagent, could form soluble aggregates by noncovalent interactions. Gezimati *et al.* (1996) recently showed that untreated BSA formed similar complexes at 75°C in the absence as well as in the presence of β-Lg, supporting the earlier suggestion that many food proteins adopt a "molten globule" conformation as an intermediate during thermal denaturation (Ptitsyn, 1995).

2.4.2.2. Aggregation of mixtures of whey proteins

Besides self-aggregation of individual whey protein, aggregates formed from interactions between different combinations of whey proteins have been studied in model systems of pure protein solutions.

The two proteins, β-Lg and α-La, interacted to form soluble aggregates mainly through a thiol-disulfide interchange reaction, as well as hydrophobic interactions (Matsudomi *et al.*, 1992; Hines & Foegeding, 1993; Dalgleish *et al.*, 1997; Gezimati *et al.*, 1997). Results from size exclusion chromatography (Hines & Foegeding, 1993), DSC (Paulsson & Dejmek, 1990) and gel filtration HPLC (Matsudomi *et al.*, 1992) showed that the rate of aggregation of α-La increased when heated in combination with β-Lg, compared with when it was heated alone. However, loss of β-Lg was not affected by α-La (Hines & Foegedling, 1993). Havea (1998) first reported the presence of disulphide-linked α-La polymers in heated (75°C) mixture of α-La and β-Lg, suggesting the formation of homogenous aggregates was catalyzed by a free thiol group from molecule of β-Lg.

A synergistic effect between β-Lg and BSA has been suggested as the loss of native β-Lg from solution at 80°C was increased by the presence of BSA (Hines &

Foegeding, 1993; Matsudomi *et al.*, 1994). BSA aggregated more rapidly than β-Lg, and the rate of BSA aggregation did not appear to be affected by the presence of β-Lg (Hines & Foegeding, 1993). At lower temperature (e.g. < 70°C), the number of β-Lg molecules that undergo the thermal transition is less than the number of BSA molecules, and hence BSA will be the dominant species in the aggregates. By contrast, each protein will form aggregates and has comparable rates of aggregation when heating at high temperature. BSA and β-Lg, therefore, formed homogenous and heterogeneous aggregates (Gezimati *et al.*, 1996; Havea, 1998).

α-La and BSA interacted upon heating at 80°C to form soluble aggregates through thiol-disulphide interchange reaction, while hydrophobic interactions were not involved (Matsudomi *et al.*, 1993). Besides α-La and BSA complexes, intermediate aggregates were formed by α-La molecules alone (Havea, 1998). However, the soluble aggregates formed by α-La and BSA had lower molecular weights than those did from BSA alone.

Limited information is available on heat-induced aggregation of mixture of β-Lg, α-La and BSA. When a mixture of pure proteins was heated (Havea, 1998), the denatured proteins interacted to form homogenous aggregate species of each protein as well as heterogeneous aggregates of all possible combinations of the proteins present. It further indicated that initially BSA formed aggregates with itself, β-Lg and α-La were involved in the aggregation process at some later stage.

2.4.2.3. Aggregation of whey proteins in WPC system

Using electrophoresis and size exclusion chromatography, the effects of heating commercial rennet WPC solutions (range of 10-120 g/kg, pH 6.8) were determined (Havea, 1998; Havea *et al.*, 1998). The aggregation was shown to start from as early as 2-4 min of heating at 75°C, consisting of β-Lg, α-La, BSA and Ig polymerised through hydrophobic interactions and disulphide cross-linkages. The extent of aggregation, the formation of the intermediate molecular weight products and the nature of the stabilising forces involved in the formation of the aggregates were concentration-dependent. Higher proportions of low molecular weight intermediate aggregate species (dimers, trimers, etc.) formed at low concentration of WPC, whereas large aggregates formed at high WPC concentration.

Ju & Kilara (1998b) investigated the effects of preheating on properties of aggregates of whey proteins by size exclusion HPLC and dynamic light scattering. When various concentrations (1-9%) of WPI solutions were heated at 80°C for 30 min, soluble aggregates were formed in 3-9% denatured WPI, whereas at low protein concentration (< 2%), proteins unfolded or denatured but most of the denatured proteins did not form the soluble aggregates. Size and content of aggregates formed increased with increases in WPI concentration prior to heating.

2.5. Heat-induced gelation of whey proteins

2.5.1. Mechanisms

The gelation of globular proteins involves initial unfolding (denaturation) of the protein molecules followed by the formation of a three-dimensional semi-ordered aggregate, the pores of which are capable of holding large amounts of water (Ferry, 1948; Doi, 1993). This two-step mechanism of thermally induced gelation proposed early has been widely accepted (Ferry, 1984).

Recently, a four-step sequence of events has been proposed in the heat-induced gelation of proteins (Aguilera, 1995). It includes denaturation (unfolding) of native proteins, aggregation of unfolded molecules, strand formation of aggregates, and association of strands and network formation. Globular proteins taking a single conformation in their native state are transformed to the unfolded or denatured state under certain conditions (e.g. heating). Interactions of exposed hydrophobic regions are responsible for the formation of aggregates, which are the basic building blocks leading to strands or pro-gels, and ultimately gels.

Denaturation of native proteins

It has been known that thermally induced gels of whey proteins require a certain degree of unfolding of proteins as a first step, but not total unfolding, which rarely occurs below 100°C. As mentioned earlier, denaturation of whey proteins usually occurs in the range of 60-80°C. Parris & Baginski (1991), using reverse-phase HPLC, reported that heat-induced unfolding of whey proteins began at 40°C and proceeded slowly to achieve 10% denaturation at 62°C. After renaturation to almost the initial state at 65°C, the percentage of denaturation followed a linear relationship

with temperature and was 95% complete at 85°C. Since opening of the molecular structure expose a large amount of reactive sites for intermolecular interactions, gelation of whey proteins is normally observed above 70°C (Aguilera, 1995).

Aggregation of unfolded molecules

Aggregation of denatured or partially denatured globular proteins in solution has been recognised as an integral part of the heat-induced gelation process, although the molecular mechanisms responsible for the formation of aggregates during and after protein unfolding are not well known. It may be considered that aggregation proceeds through a sequence of unimolecular unfolding reactions and bimolecular association steps to yield higher-order polymeric structures, as is the case of folding of native proteins (Aguilera, 1995). It is generally assumed that the driving forces for aggregation are nonspecific interactions between hydrophobic regions of unfolded polypeptide chains, but hydrogen bonding and ionic interactions are likely to participate as well. Stabilisation of soluble aggregates may even proceed through disulphide crosslinking between polypeptide chains (Aguilera, 1995).

Strand formation of aggregates

The most outstanding microstructureal feature of aggregate gels made from whey proteins is the presence of a homogeneous network of connected protein particles or aggregates forming a three-dimensional matrix with interstices filled by a liquid or aqueous solution (Aguilera, 1995). Doi (1993) presented two models for the formation of globular protein gels from aggregates: random clustering, usually referred to as "particulated" gels, and a "string of beads", usually referred to as "fine stranded" gels. β-Lg, BSA and whey protein products are known to form either type of gel, depending on the pH and ionic strength (Stading & Hermansson, 1991). The network of aggregated or particulate gels is composed of more or less spherical particles linked together, forming the strands of the network.

Association of *strands and network formation*

Protein gels are usually divided into physical gels and entangled networks. The former are stronger gels, while the latter are formed by the topological entanglement

of chains and gelled networks at frequencies higher than some typical entanglement lifetime or are viscoelastic liquids without an equilibrium modulus. Whey protein gels appear to be physical gels of infinite molecular weight (Aguilera, 1995).

2.5.2. Gel properties of whey proteins

Heat-induced gelation of whey proteins, as one of important functional properties, has been studied thoroughly (Mulvihill & Kinsella, 1988; Paulsson & Dejmek, 1990; Aguilera, 1995; Gezimati *et al.*, 1996, 1997; Havea *et al.*, 1998). A wide range of techniques is used in studying gels. The gel point, which can be defined as the point on a temperature scale where the sol-gel transition in a polymer solution occurs, is usually determined using rheological measurement (Tang *et al.*, 1993, 1995). Rheological measurement is useful in gelation studies because it measures various viscoelastic properties which are related to different gel properties including storage modulus (G'), loss modulus (G") and phase angle (tan δ) (Tang *et al.*, 1993). Furthermore, this technique can measure the development of these properties during the heating of a protein system. Gel strength is usually measured by compression techniques, in which the force required to compress a well-defined size piece of gel is used as a measure of gel strength.

Generally, whey proteins can form two types of heat-induced gels (Doi, 1993). The first is an aggregated gel, which comprises relatively large particles (> 250 nm) bound to one another to form a network. These gels are usually opaque because of relatively large aggregates and exhibit low water-holding capacity. The second category is the fine-stranded gels, produced by association of small-diameter molecules to form an order network. These gels are usually clear and exhibit good water holding. Variation between these two groups exists and mixed gels can also be formed (Barbut, 1996). As the denaturation of the proteins is affected by many factors, the properties of whey protein gels are also affected by factors such as type of protein, protein concentration, temperature, pH, ionic strength and presence of other ingredients such as lactose. The structure of protein gels can vary widely depending on these conditions, and has an impact on gel properties, such as rheological properties, sensory qualities and water-holding capacity.

2.6. Cold-set gelation of pre-denatured whey proteins

Traditionally, foods containing whey protein ingredients have to be heated above 65°C before the proteins would form gels or thicken solutions, which limited their application in many types of food products. The gel formed at room temperature or at incubation temperature with such additives as salts, acidulates or proteases are called cold-setting or set gelation (McClements & Keogh, 1995). Such cold-set gelation required an initial preheat step to denature whey proteins, followed by incubation. The gel properties depended on preheating conditions as well as factors affecting denaturation and aggregation of whey proteins and affecting gel induction.

Few reports have been published on cold-set gelation of WPI solution by addition of salts (Barbut & Foegding, 1993; McClements & Keogh, 1995; Hongsprabhas & Barbut, 1997a, b; Ju & Kilara, 1998a, b), acidification (Kawamura *et al.*, 1993; Ju & Kilara, 1998a) or enzymatic hydrolysis (Sato *et al.*, 1995; Ju *et al.*, 1997). These studies mainly focused on the effects of preheat treatment or denaturation levels, protein concentration, pH and salt concentrations on rheological and textural properties of cold-set gels. Although the information on cold-set gelation is limited as compared to that on heat-induced gelation, the results have showed that cold-set gels are different to heat-induced gels and the gel properties depend on preheating, the rate of denaturation and the extent of aggregation, as well as factors affecting the gel incubation of whey proteins.

2.6.1. Formation of cold-set gelation

The production and application of cold-setting whey protein ingredients involves two stages: (i) the preparation of a heat-denatured protein solution and (ii) the induction of gelation at low temperatures. This requires careful control of the initial solution conditions (pH, mineral content and protein concentration) and heating conditions (temperature and holding time).

The pre-denatured whey protein solution can be obtained by heating the solution below the critical protein gelling concentration, but above the denaturation temperature of the whey proteins, at neutral pH and in the presence of little or no salt (Ju & Kilara, 1998b). Typically, solutions are held for between 5 and 60 min at temperatures between 70 and 90°C, to ensure the correct degree of protein unfolding

and aggregation. The pH of the initial solution should be sufficiently different from the isoelectric point of the proteins to ensure that they do not immediately aggregate upon heating. Most experiments in the literatures have been carried out at pH 7.0 (~ 2 pH units above the isoelectric point of the proteins) (Barbut & Foegding, 1993; McClements & Keogh, 1995; Hongsprabhas & Barbut, 1997a, b; Ju & Kilara, 1998a,b). The salt concentration of the initial solution must be low enough to prevent excessive aggregation of the protein molecules. In addition, the protein concentration must be low enough to prevent the molecules from forming a three-dimensional network, i.e. gel.

After a solution of heat-denatured whey proteins has been prepared, it can be cooled and made to thicken or gel by adding salts ($CaCl_2$, NaCl), acidulant or by enzyme hydrolysis at room temperature or at incubation temperatures.

2.6.2. *Rheological and textural properties*

There was an agreement that cold-set gels from the denatured WPI solution had fine-stranded microstructures (Barbut & Foegeding, 1993; McClements & Keogh, 1995; Barbut, 1996; Hongsprabhas & Barbut, 1997a; Ju *et al.*, 1997; Ju & Kilara, 1998b), but opaque and semi-opaque appearances were also observed (Barbut & Foegeding, 1993; Kawamura *et al.*, 1993; Ju & Kilara, 1998a). The WPI cold-set gel exhibits higher gel strength and water-holding capacity (WHC) compared to a heat-induced gel produced with the same amount of 10 mM $CaCl_2$ (Barbut & Foegeding, 1993). The same approach can be used with Na^+, where adding 200 mM NaCl to pre-heated WPI suspensions results in less turbid gels with higher complex modulus (G*), compared to gels formed with an equal Na^+ present during heating (McClement & Keogh, 1995).

Ju *et al.* (1997) reported that higher denaturation (%D) resulted in earlier gelation and a faster increase in gel firmness. The relationship between %D and the rate of gel firming was almost linear. The gel from highly denatured WPI (98% denaturation) had a fine-stranded structure, whereas the gel from lower denatured WPI had a particulate microstructure.

The networks of two gels induced by $CaCl_2$ and GDL were built with similar thickness of fine-stranded aggregates, indicating that salt and acid induced gelation by

promoting interactions of the aggregates that originally existed in the denatured WPI solution (Ju & Kilara, 1998b). It was further noted that the fine-strands in the cold-set gels were originally present in denatured WPI solution. This suggested that the effects of salts, acidulant, or enzymes were to connect preformed thermal aggregates into a gel network (Ju & Kilara, 1998a). Results of comparing enzyme-induced gels formed from pre-denatured and unheated whey proteins strongly suggested that the type of gel formed from denatured whey proteins was determined by the type of aggregates formed at the early stages of gelation (Ju *et al.*, 1997).

In conclusion, the properties of cold-set gels of WPI were dependent on the denaturation and aggregation during preheating. Certain degrees of unfolding and formation of "soluble" aggregates of whey proteins were essential for cold-set gelation. Heating time, temperature and protein concentrations affected denaturation level, aggregate size and numbers, which then lead to formation of different type of gels by addition of salts, acidulant, or enzymes. The research results suggest that it is possible to manipulate preheated protein concentration or amount of heat to control the extent of denaturation, the size and content of soluble aggregates to develop new whey protein products for application of cold-set gelation.

2.7. Factors affecting denaturation, aggregation and gelation of whey proteins

2.7.1. Protein concentration

2.7.1.1. Effects on denaturation and heat-induced gelations

The influence of protein concentration on structural changes of β-Lg has been studied using a combination of techniques (Iametti *et al.* 1995). The irreversible modification of the tertiary structure was not concentration dependent, while the temperature required for the occurrence of protein swelling, the initial step in the formation of associated forms of the protein, increases with the protein concentration. On heating at 80°C, the rates of denaturation of the individual whey proteins in skim milk increased with total protein concentration and, to a lesser extent, with increasing whey protein concentration (Law & Leaver, 1997). Havea *et al.* (1998) examined rennet WPC solutions, heated at 75°C, at a range of concentrations (10-120 g/kg, pH 6.8). The results revealed that the extent of protein aggregation and the formation of the intermediate molecular weight products were concentration-dependent, which was

in line with the result of Nielsen *et al.* (1996), who found the extent of aggregation of β-Lg isolated from acid whey increased with protein concentration and heating time (at 75°C).

The hardness of heat-induced protein gels is affected by the concentration and purity of protein. The minimum amount of protein required to form a gel is an important criterion for the gel forming abilities and is dependent on the environmental conditions such as pH, ionic strength, heating temperature and time. Paulsson *et al.* (1986) found that minimum protein concentrations required for gelation at pH 6.6 in the presence of 1% NaCl were 1% and 2% for BSA and β-Lg, respectively, in the temperature range of 60°C-90°C. As the protein concentration is increased, the number of potential interactions between the protein molecules are enhanced resulting in increased gel strength, reduced gelling time and finer gel networks (Paulsson *et al.*, 1986; Matsudomi *et al.*, 1991).

2.7.1.2. Effect on cold-set gelations

In the heat-denatured protein solution, the protein concentration has a major influence on the properties of the viscous solutions or cold-set gels (Kitabatake *et al.*, 1996; Ju & Kilara, 1998b). At relatively low protein concentrations, the heat-denatured protein will tend to form a viscous solution rather than a gel (Kitabatake *et al.*, 1996). As the protein concentration is increased, the viscosity of this solution increases. Above a critical protein concentration, the heat-denatured protein solution forms a gel rather than a viscous solution.

Using size exclusion HPLC, dynamic light scattering (DLS) and texture analyzer, Ju & Kilara (1998b) found that whey protein concentration during preheating had a remarkable effect on aggregation and gel formation. Preheating WPI of low protein concentration (< 2%) at 80°C for 30 min unfolded or denatured the protein, but most of the denatured protein did not form the soluble aggregates. In contrary, soluble aggregates formed in 3-9% denatured WPI solution. Both aggregate size and content increased with increasing whey protein concentration (Table 2.4). Native WPI solution, for instance, measured 8 nm of mean particle size, whereas 9% denatured WPI had 61 nm mean aggregate size, reflecting a 7.6-fold increase in mean particle size from that of native whey protein.

Table 2.4. Aggregates formed at different preheated whey protein concentrations

Preheated	Aggregate size		Aggregated
Concentration (%)	By SE-HPLC (MW)	By DLS (nm)	whey protein (%)
3	~ 103×10^4	21	34
5	~ 125×10^4	-	56
7	≈ or > 1000×10^4	-	90
9	≈ or > 1000×10^4	61	97

The 1-2% pre-denatured WPI solution did not form self-supporting gels upon addition of $CaCl_2$ (20 mM) and GDL (glucono-δ-lactose, 0.6%, w/v), suggesting the formation of soluble aggregates was a necessary step in forming cold-set gel (Ju & Kilara, 1998b). Gels formed at protein concentration ≥ 3%, linearly increased in hardness with increased protein concentration prior to heating or with the aggregate size and content during preheating. For example, the gel (3% whey protein) from dilution of the 9% preheated WPI solution was > 2 times stronger than the gel (3% whey protein) from the 3% preheated WPI solution. Hongsprabhas & Barbut (1997b) also investigated the effects of protein concentration on Ca^{2+}-induced cold gelation of WPI. Increasing whey protein concentration (from 6 to 10% w/v) prior to heating decreased gel opacity but increased gel strength and WHC.

In addition to pre-denatured whey protein concentration, the concentration for setting cold gelation also affected the gel properties. Earlier study showed that the WPI required 12% whey protein to form a self-supporting gel after heating 80°C for 30 min. However, by first heat-denaturing 9% WPI solution (80°C, 30 min), gelation occurred at 1% whey protein with addition of GDL (0.8%, w/v) or $CaCl_2$ (20 mM), and at 2% or 3% whey protein with addition of *Bacillus licheniformis* protease (BLP, E:S = 1%) or NaCl (200 mM), indicating the procedure of cold-set gelation could enhance the functionality of whey proteins (Ju & Kilara, 1998a).

Hardness of cold-set gel showed non-linear increases with the concentration of whey protein (1-8% whey protein diluted from 9% pre-denatured WPI). Gel hardness increased slowly at lower whey protein concentrations and more rapidly at higher

whey protein concentrations, which was similar to heat-induced whey protein gelation (Ju & Kilara, 1998a).

2.7.2. Temperature and heating time

2.7.2.1. Effects on denaturation and heat-induced gelations

Reversible changes in whey protein structures occur mainly at temperature up to 60°C as these reactions are governed mainly by hydrophobic association which is enhanced as the temperature increases up to 60°C and above this temperature the changes become irreversible (de Wit & Klarenbeek, 1984). Denaturation of whey proteins in rennet casein whey increased slowly on heating 60°C and 70°C, but proceeded rapidly between 80°C and 90°C (Donovan & Mulvihill, 1987). The denaturation of whey proteins occurred more rapidly and completely at 71°C than at 66°C when a WPC solution was heated for 120 min (Hollar *et al.*, 1995). Oilfield *et al.* (1998) also indicated that the extent of α-La and β-Lg denaturation and association in skim milk increased with an increase in both heating time and temperature.

Gelation is heating temperature and time dependent. Heating protein solutions above the minimum denaturation temperature of the constituent proteins are required for gel formation (Matsudomi *et al.*, 1991), although heating at temperature below the denaturation temperature may also result in gelation but requires longer heating time before any significant structures begin to develop. The effects of heating temperature and time are also dependent on the protein type and concentration. When other factors are maintained, gel hardness increases with increasing heating temperature and time.

Heating rate also affect the gelation process. Rapid heating does not allow enough time for proteins to unfold and aggregate in a sequential manner even if the temperature is above the denaturation temperature while slow heating allows proteins enough time for unfolding and aggregation resulting in much stronger gels (Standing & Hermansson, 1990).

2.7.2.2. Effects on cold-set gelations

The effects of heating time and temperature on whey protein unfolding (Mc Clements & Keogh, 1995), denaturation (Ju *et al.*, 1997; Ju & Kilara, 1998a), aggregation (Ju & Kilara, 1998b) and rheological properties of cold-set gels (Barbut

& Foegeding, 1993; Hongsprabhas & Barbut, 1996; Ju & Kilara, 1998b) have been investigated. It was demonstrated that a high degree of denaturation induced a fine-stranded network formation, which exhibited high gel strength and WHC.

Barbut & Foegeding (1993) established that preheating WPI solution to ≥ 70°C was required for cold gelation to occur in ≤ 14 h when dialysed against $CaCl_2$ solutions. McClements & Keogh (1995) monitored unfolding of protein molecules using DSC, maximum unfolding occurred at 72 ± 1°C when a 10% WPI solution was heated from 30 to 100°C. The process of gelation was strongly dependent on the extent of protein denaturation (%D), which was a non-linear function of heating time (Ju *et al.*, 1997). The total %D, calculated from the HPLC profiles, after 30 min of heating at 80°C was 98%, which was confirmed by Ju & Kilara (1998a) under similar heating conditions, who observed that nearly all native whey protein in WPI solution formed soluble aggregates after 30 min heat treatment.

A requirement of minimum aggregation level was necessary for cold gelation. (Ju & Kilara, 1998b). Heating 8% WPI for 4 min only resulted in 46% native proteins converting to aggregates, and the aggregates were ~ 64×10^4 MW. This preheated WPI solution did not gel upon addition of $CaCl_2$ or GDL. After 5 min of heating, 62% of the native protein formed aggregates with a larger MW of 94×10^4. This pre-denatured WPI solution gelled upon addition of $CaCl_2$ or GDL. The longer time and higher temperature of heating could lead to higher MW and higher content of aggregates (Barbut & Foegeding, 1993; Ju & Kilara, 1998b). The small differences in the initial state of aggregation of the unfolded protein molecules might have a large effect on the ridigity of the gel produced when salt was added (McClements & Keogh, 1995). The enlarged and concentrated aggregates led to the formation of harder gels. High pre-heating temperature (90°C) produced clearer gels with high WHC and gel extensibility (after compression) than low temperature (70°C) (Hongsprabhas & Barbut, 1996)

The rate of gel formation increased on cooling, suggesting the major driving force for protein aggregation and gelation was the hydrophobic effect. Once formed, the rigidity of the gels decreased with decreasing temperature, which suggested that non hydrophobic forces (e.g. hydrogen bonding, van der Waals forces and disulphide

bonds) were more important in determining the final gel rigidity (McClements & Keogh, 1995).

In addition to preheating temperature, gelation temperature also has an important influence on viscosity and gel characteristics of pre-denatured WPI solutions (Kitabatake *et al*, 1996; Hongsprabhas & Barbut, 1997a). The viscosity of WPI measured at 38.3 sec^{-1} generally decreased as temperature increased from 0°C to 37°C at various NaCl concentrations (Kitabatake *et al*, 1996). With the exception of gels formed at 1°C with 10 mmol/L $CaCl_2$, the increase in gelation temperature (from 1°C to 11 and 24°C) resulted in large aggregate (more opaque gels), which decrease gel clarity, gel strength and WHC (Hongsprabhas & Barbut, 1996; 1997a). This suggested that the gel characteristics could be modified by controlling the conditions both during protein denaturation (i.e. pre-heating) and gel formation (i.e. gelation temperature) separately.

2.7.3. pH

Thermal denaturation and aggregation of whey proteins are markedly pH-dependent. Individual whey proteins exhibit different responses to pH, probably due to different content and distributions of polar residues (Donovan & Mulvihill, 1987). In an extensive study on the effect of pH on the thermal stability of whey proteins in rennet casein whey between 4.5 and 7.0, Donovan & Mulvihill (1987) found that β-Lg is most sensitive to denaturation at pH 6.7, BSA is most stable, while α-La seems to be relatively independent of pH in the pH range studied. However, Hillier *et al.* (1979) indicated that α-La had a slower rate of denaturation when heated cheese whey at pH 4.0 than at pH 6.0.

Boye *et al.* (1997) reported that the aggregates of β-Lg formed were very large (1-2 μm) and globular at acid pH (3.5) but much smaller and amorphous at alkaline pH (8.6). By combination of size-exclusion chromatography with laser-light scattering, Hoffmann & van Mil (1999) found that the rate of conversion increased strongly with pH (range 6.4 –8.0), whereas the molecular mass of the aggregates decreased strongly. The aggregates were formed mainly by intermolecular disulphide bonds, but even at pH 6.0, at which very large noncovalently linked aggregates were formed, also thiol/disulphide exchange reactions were involved.

Since pH affects molecular conformation and intermolecular interactions, it is not surprising that it influences gel network structure and rheological characteristics. Several investigations on whey protein gelation have found that opaque gels are formed at pH 4-6, while above and below this range gels are translucent (Stading & Hermansson, 1991; Langton & Hermansson, 1992). Translucent whey protein gels have fine-stranded microstructures and the rheological properties are pH-dependent. Fine-stranded gels formed at pH < 4 are weak (low values for fracture stress) and brittle (low values for fracture strain). In contrast, fine-stranded gels formed at pH > 6 are strong and rubbery, with high fracture stress and strain values (Stading & Hermansson, 1991; Tang *et al.*, 1995). Shimada & Cheftel (1988) noted a decreased firmness of WPC gels and an increase in their elasticity from pH 6.5 to 9.5. Gels formed at pH 7 to 9 were more elastic and less coagulated than gels formed at low pH (< 6.0).

Gelation occurred when heat-denatured WPI solutions were incubated at 20-50°C in the presence of GDL, which changed pH below 5.8 (Kawamura *et al.*, 1993; Ju & Kilara, 1998a). Varying concentrations of GDL (0.2-2.0% w/w), resulted in various pH values in the final gels, greatly affected the texture properties of formed gels (Ju & Kilara, 1998a). Translucent gels were obtained at addition of 0.4% GDL (pH 5.3) at 45°C. Further increased GDL concentration led to formation of white opaque gels. Maximum gel hardness occurred at pH 4.7 or at 0.8% GDL. Increasing or decreasing GDL concentration from 0.8% decreased gel hardness. The effect of varying GDL concentrations on adhesiveness showed similar trend as that on gel hardness. However, maximum adhesiveness occurred at pH 4.4 instead at pH 4.7 for gel hardness. Change of pH by the addition of GDL had little effect on gel cohesiveness. The pH-induced gels had a low and almost constant cohesiveness from pH 5.3 to 3.5 (Ju & Kilara, 1998a).

2.7.4. Salt type and concentrations

The milk salts have a significant effect on the heat-induced denaturation and aggregation of whey proteins. Varunsation *et al.* (1983) showed that the effects of Ca^{2+}, Mg^{2+} and Na^{+} were to promote the denaturation and aggregation of whey proteins, but only on the alkaline side of the isoelectric point (> pH 5.5). The effect of

Ca^{2+} was greater than that of Mg^{2+} but both divalent cations affected protein denaturation and aggregation to a much greater extent than Na^{+}. Removal of Ca^{2+} by EDTA decreased the denaturation temperature of α-La by 20°C, suggesting that binding of α-La to Ca^{2+} stabilise its tertiary structure. The denaturation temperature of WPC increased from 76.3°C in the absence of NaCl, to 85.3°C with the addition of 2M NaCl (Boye *et al.*, 1995).

Aggregation of β-Lg was very sensitive to small variations in ionic strength and ionic composition of the medium (Hoffmann *et al.*, 1996). The particle size increased with salt concentration in the range studied (up to 20 mM-NaCl and 1.0 mM-$CaCl_2$), demonstrating that salt led to the formation of large polymer particles. At high NaCl concentration, physical bonding became increasingly important and large aggregates that continue to grow in time were formed (Verheul *et al.*, 1998). These results were consistent with the findings of Eloffson *et al.* (1996) and Hoffmann *et al.* (1997), who detected the average molecular masses of aggregates formed with β-Lg.

Ca^{2+} has also been suggested to be involved in the formation of large "insoluble" aggregates (Parris *et al.*, 1993, 1997; Hollar *et al.*, 1995). As Ca^{2+} decreased, WPC solution formed more soluble aggregates and few "insoluble" precipitates, the amount of α-La relative to the β-Lg aggregates associated with the soluble aggregates also increased (Parris *et al.*, 1997).

Schmidt *et al.* (1978) determined that the mineral components have a significant influence on the gel characteristics of WPC. Maximum gel hardness values of dialysed WPC are attained when protein suspensions contain 200 mM NaCl or 11.1 mM $CaCl_2$. Mulvihill & Kinsella (1988) supported these observations with their findings that hardness values of β-Lg gels maximised at 200 mM NaCl or 10 mM $CaCl_2$. Kuhn & Foegeding (1991) noted WPI formed translucent, gelatin-like gels with low levels of NaCl (25-30 mM) and opaque curd-like gels with 7.5 mM $CaCl_2$. Increasing levels of either NaCl or $CaCl_2$ increases gel strength, shear stress and other rheological properties until maximum values are reached, and then decrease with higher salt concentrations (Schmidt *et al.*, 1978; Mulvihill & Kinsella, 1988; Kuhn & Foegeding, 1991).

The effect of Ca^{2+} or Na^{+} level on Ca^{2+} or Na^{+}-induced cold gelation of WPI has been investigated (Barbut & Foegding, 1993; McClements & Keogh, 1995; Barbut &

Drake, 1997; Hongsprabhas & Barbut, 1997a, b; Hongsprabhas & Barbut, 1998; Ju & Kilara, 1998a). Varying concentrations of $CaCl_2$ (5-40 mM), NaCl (20-400 mM) greatly affected gelation of the denatured WPI solution and texture properties (hardness, adhesiveness, and cohesiveness) of formed gels (Ju & Kilara, 1998a). Low concentration of $CaCl_2$ (5 mM), NaCl (20 mM) did not cause gelation of 8% denatured WPI solution (Ju & Kilara, 1998a).

According to Ju & Kilara (1998a), increasing $CaCl_2$ concentration continuously enhanced gel hardness, which was in line with the result of Barbut & Foegeding (1993), who reported that increasing $CaCl_2$ concentration (10 mM to 150 mM) progressively increased shear stress of $CaCl_2$-induced gels. At a low $CaCl_2$ concentration (10 mM), a clear gel was formed. Scanning and transmission electron micrographs revealed the formation of a fine protein strand microstructure (Barbut, 1995). More opaque gels progressively produced when $CaCl_2$ concentration was increased, indicating that large aggregates were formed which was associated with a significant reduction in WHC (Barbut, 1995; Hongsprabhas & Barbut, 1997a). Shear stress at fracture increased as the $CaCl_2$ level increased up to 180 mmol/L and then leveled off. The WHC and shear stress values of the cold set gels were significantly higher than that of the heat-induced gels (Barbut, 1995).

Ju & Kilara (1998a) observed that addition of 50 mM NaCl resulted in gelation of 8% WPI solution diluted from 9% heat-denatured. The level of NaCl was lower than that from Barbut & Drake (1997), who reported that a minimum of 75 mM NaCl was required to induce cold gelation in 10% WPI suspensions. Increasing NaCl concentration quickly increased gel hardness and maximum gel hardness occurred at 200 mM NaCl. (Nakamura *et al.*, 1995; Ju & Kilara, 1998a). Mulvihill & Kinsella (1988) reported a similar observation for heat-induced gels in the presence of NaCl. More additions of NaCl up to 400 mM slowly decreased gel hardness. The gels, formed by gradually increasing NaCl concentration, showed a progressive decrease in WHC which plateaued at 300 mM (Barbut, 1996)

2.8. Conclusions

WPC has been used in a wide range of food applications because of its highly nutritional value and desired functional properties such as heat-induced and cold-set

gelation ability. It has been generally accepted that the functional properties of WPC are affected not only by processing history, but also by the composition and quality of the product. The quality of whey proteins is primarily determined by the amount of denaturation during the processing. For example, two WPC products with exactly the same composition can exhibit very different functional properties due to differences in their degree of denaturation. Extensive studies, therefore, have been conducted to investigate thermal denaturation and aggregation of whey proteins as well as environmental conditions, which influence denaturation and aggregation of whey proteins. The mechanism of thermal denaturation and aggregation formed by individual and mixture whey proteins has been thoroughly researched. A considerable amount of information has been established on effects of several factors such as heating temperature and holding time, pH, protein concentration and ionic strength on heat-induced denaturation, aggregation and gelation.

The denaturation degree, the size and type of protein aggregates formed during heat treatment can be manipulated by controlling the heating conditions. It is possible, therefore, to develop a novel process for producing WPC products containing pre-aggregated whey proteins to achieve desired gelation properties. However, information related to properties of cold-set gelation of pre-denatured whey proteins is limited. Especially there is no publication on factors affecting cold-set gelation of pre-denatured whey proteins in WPC system.

To optimise processing procedures and achieve desirable product functionality, it is helpful to have more detail information on the effects of environmental conditions on the size of the aggregates formed and on the gel induction. It is desirable to permit optimisation of preheating that ensure the formation of specially sized and shaped aggregates, which give the desired functional characteristics to the final WPC products.

CHAPTER 3
MATERIALS AND METHODS

3.1. Materials

3.1.1. Samples

Acid whey and UF retentate were obtained from Anchor Products, Edgecumbe, and kept at -30°C. WPC powders were produced from UF retentate in the pilot plants at Massey University and Anchor Products, Edgecumbe.

3.1.2. Chemicals

All chemicals used for the preparation of electrophoresis buffers (obtained from Bio-Rad Laboratories, Richmond, USA) were of analytical grade. Glucono-δ-lactone (GDL), NaCl and $CaCl_2$ were purchased from Sigma Chemical Co. (St. Louis, MO). Water used in sample preparation and electrophoresis was purified using a Milli-Q system (Millipore Corporation, Bedford, USA).

3.2. Methods

The present study consisted of three stages: preliminary experiments, pilot plant trials and functional property testing (Figure 3.1). The bench scale preliminary experiments investigated the effects of protein concentration, heating temperature and time, and pH on denaturation and aggregation of whey proteins in acid whey and UF retentate using PAGE analysis. The pilot plant trials were carried out to test the heating conditions for denaturing whey proteins to produce heat-denatured and unheated WPC powders at Massey University and Anchor Products. The viscosity or gel strength of the heat-denatured or unheated WPC solutions with or without additives was then measured by a viscometer or an Instron.

3.2.1. Composition analysis

The protein contents of acid whey, UF retentate and WPC powders were determined by the Kjeldahl method with a nitrogen conversion factor of 6.38 (AOAC, 1984), using Kjeldahl 1007 Digestor and Kjeltec 1026 Distilling unit (Tecator, Sweden). Total solids were calculated from the moisture content that was determined by oven-drying of pre-weighed duplicate samples at 105°C for 24 hrs, cooling in a

desiccator for 2 hrs and then reweighing the samples. The ash content was determined by reweighing the samples after ashing in a muffle furnace at 550°C overnight and cooling in a desiccator. All the analyses were carried out in duplicate or triplicate.

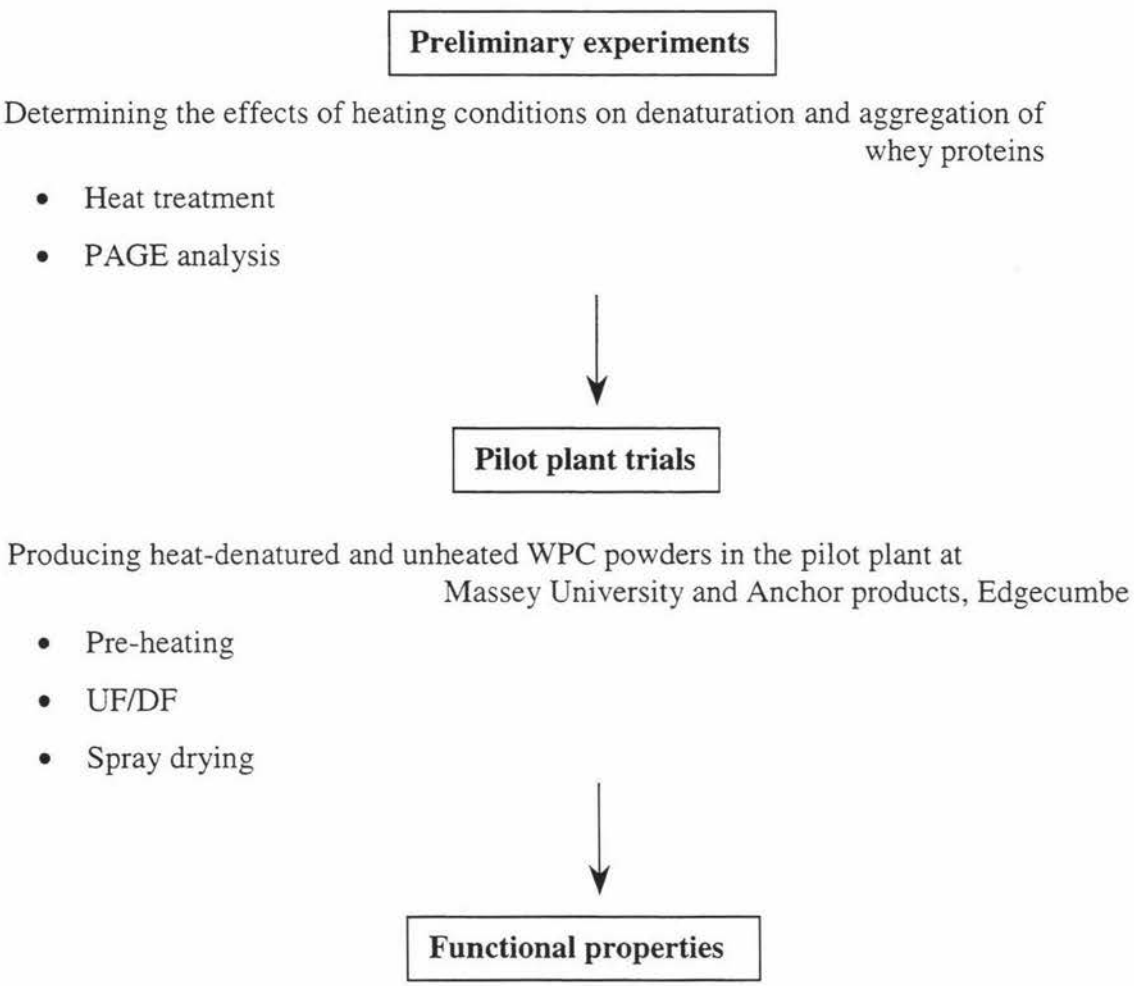


Figure 3.1. Schematic diagram of experimental procedures for the present study.

3.2.2. pH measurement

The pH of acid whey, UF retentate and WPC solution was measured by Orion 720A pH meter (Orion Research, Boston, MA 02129 USA), which was first calibrated using standard solutions of pH 4.0 and pH 7.0 at 20°C. The temperature knob of the pH meter was set to corresponding temperatures when pH of solutions at 5°C, 20°C, 30°C or 40°C was measured.

3.2.3. Heat treatment of acid whey and whey protein solutions

Acid whey, whey protein solutions (diluted UF retentate with Milli-Q water to protein concentrations of 1%, 2%, 5% or 10%) or WPC solution (diluted to protein concentration of 1%) was adjusted to pH 7.0 or 7.5 with 2M NaOH. Aliquots (3 ml) of samples were then placed in glass tubes with screw caps (12 mm i.d. × 100 mm) and heated at 80°C or 95°C in a thermostatically controlled water bath for various times (1-60 min). Immediately after heating, the tubes containing the samples were immersed in ice water for 5 min and held at room temperature for 2 hrs before further analysis.

3.2.4. Polyacrylamide gel electrophoresis (PAGE) analysis

The heated or unheated whey protein solutions were diluted with the appropriate sample buffer and analysed by native PAGE, using Mini-Protein II dual slab cell system (Bio-Rad Laboratories) as described by Havea (1998).

3.2.4.1. Preparation of resolving gel

The native resolving gel was made from a mixture of 8.0 ml of a 37.5:1 mixture of acrylamide and N,N'-methylene-*bis*-acrylamide, 2.0 ml of resolving gel buffer (3.0 M Tris adjusted to pH 8.8) and 6.0 ml of water. This mixture was degassed by evacuation while stirring. Just prior to pouring the gel, 80 µl of freshly prepared 10% (w/v) ammonium persulphate solution and 8 µl of N,N,N',N'-tetramethylethylenediamine (TEMED) were added to the mixture.

The gel setting apparatus was assembled using 0.75mm spacers and 3.3 ml of resolving gel solution was put between each pair of glass plates. About 0.5 ml of

water was then placed above the resolving gel solution and the apparatus was set aside about 30 min at room temperature to allow acrylamide solution to polymerise gel. The water was then drained off with filter paper before pouring the stacking gel.

3.2.4.2. Preparation of stacking gel

The stacking gel was made from a mixture of 1.0 ml of a mixture of acrylamide and bis-acrylamide, 2.0 ml of stacking gel buffer (0.5 M Tris adjusted to pH 6.8) and 5.0 ml of water. Following the degassing, 40 µl of 10% ammonium persulphate solution and 8 µl of TEMED were added. This mixture was then carefully pipetted on the top of the resolving gel and the slot-formers (plastic comb) was immediately inserted, taking care that no air bubbles were entrained. Once the gel was set, the gel-formers and gels were often enclosed in a plastic bag which was kept at 5°C.

3.2.4.3. Running gels

The electrode buffer stock solution was 0.125 M Tris and 0.95 M glycine (adjusted pH to 8.3). In use, this solution was diluted 1:4 with water. Sample was diluted with sample buffer and applied into sample well using a syringe. The sample buffer comprised 20% stacking gel buffer, 0.01% bromophenol blue and 8% glycerol. The maximum voltage, current and power were set at 210 V, 70 mA and 6.5 W, respectively, on the power supply (Bio-Rad model 1000/500). For running two gels, the approximate running time was 1 h when the tracking dye disappeared from the bottom

3.2.4.4. Gel staining and destaining

Each gel was stained for 1 h in 50 ml of Coomassie dry solution (0.1% Coomassie blue R-250, 25% isopropanol, 10% acetic acid in water) in a closed 500 ml container with continuous agitation. Following the staining, gel was destained with 100 ml of destaining solution (10% isopropanol, 10% acetic acid) for 1 and 19 h, respectively. After that, the destaining solution was replaced with water and the gels were kept for later use.

3.2.4.5. Gel analysis

After staining and destaining, the gels were scanned on an Ultrascan XL laser densitometer (LKB Produkter AB, Stockholm-Bromma, Sweden) and the results were analysed using an LKB 2400 GelScanXL software program to obtain quantitative results of major proteins. The peak area of each protein band was reported as a percentage of the corresponding band in the unheated control samples.

The gels were then photographed using a 35 mm camera fitted with both green (XI) and orange (G) Hoya filters to minimise the stray light on to 100 ASA Kodak T-max film, which was then processed in the usual way.

3.2.5. Production of WPC powders at Massey pilot plant

A simplified schematic flow diagram of the production of both heat-denatured and unheated WPC powders is shown in Figure 3.2. The original UF retentate which was used as raw material for production of WPC product was described as primary retentate (1° retentate), whereas the resulting retentate from the original retentate through a second UF/DF process was described as secondary retentate (2° retentate). The processes, including heat treatment, UF/DF and spray drying, were applied for producing denatured WPC powders in trial 1 and trial 2 in the pilot plant at Massey University. The unheated WPC powders, used as control samples, were produced from trial 3 and trial 4 using the same procedure except the heat treatment prior to UF.

3.2.5.1. Heat treatment of whey protein solution prior to UF/DF

The UF 1° retentate was diluted with deionised water to give 1% protein solution and the pH was adjusted to 7.0 with NaOH. The solution was heated to 80°C by pumping through a steam jacketed holding tube of ultra-high-temperature (UHT) plant. Following the heat treatment in the UHT plant, the heated whey protein solution was poured into a hot water jacketed pan and held at 80°C for 20 min. The denatured retentate solution was then immediately poured into containers that were surrounded with ice, and cooled to 50°C within 10 min.

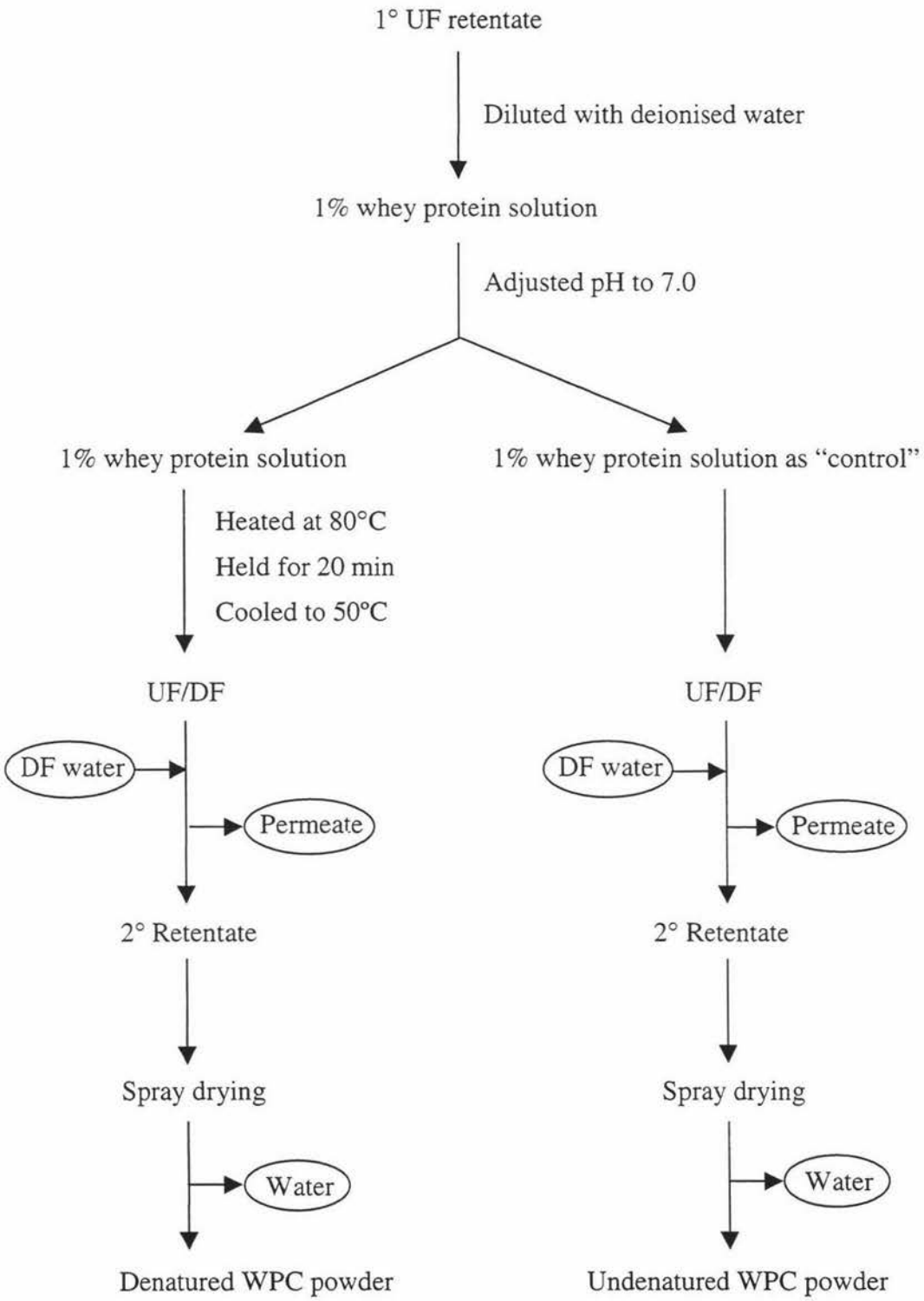
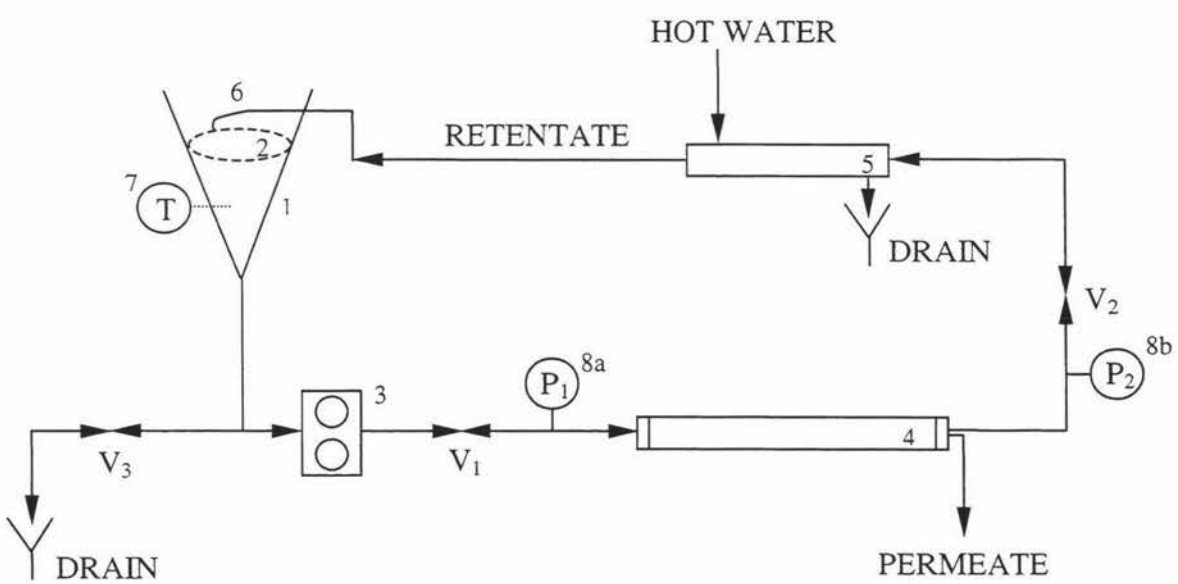


Figure 3.2. Process flow chart for production of WPC at the Massey pilot plant.

3.2.5.2. UF/DF

Figure 3.3 shows the schematic diagram of the UF pilot plant at Massey University. The 1% whey protein solution diluted from 1° retentate (heated or unheated) was placed in the conical feed vessel and pumped at P_1 and P_2 of 140 and 40 kPa, respectively, into the UF unit (HFM 100; Abcor Inc., USA). The permeate was collected in a measuring cylinder, while the retentate stream was returned into the feed vessel. The circulation was maintained at 50°C by hot water heat exchanger. DF deionised water (15% of initial volume of the solution) was added to the vessel after 80% of the initial volume was collected as a permeate. The circulation was continued until 90% of initial volume was collected as permeate. The resulting 2° retentate, containing approximately 10% protein, was collected to dry.



1. Conical balance tank
2. 0.5 mm sieve screen
3. Positive displacement pump
4. Koch separating module
5. Shell and tube heat exchange
6. Flexible hose return line
7. Temperature gauge

8a, 8b. Pressure gauge

V_1, V_2, V_3. Butterfly valves

Figure 3.3. Schematic diagram of KOCH-UF Pilot Plant at Massey University.

3.2.5.3. Spray drying

The 2° retentates from UF/DF were spray dried using a Lab Spray Drier (Anhydro, Copenhagen, Denmark) to produce WPC powders in trial 1-4. The spray drier was fitted with nozzle atomisation. The inlet and outlet air temperatures used were 225°C and 80°C, respectively. The feed rate of sample solutions was adjusted to control the inlet air temperature.

3.2.6. Production of WPC powders in industrial pilot plant

Following similar process scheme shown in Figure 3.2, the manufacture of WPC powders, trial 5-7, was carried out using pilot scale UF/DF plant and Spray Drier at Anchor Products, Edgecumbe (Figure 3.4).

3.2.6.1. Heat treatment of whey protein solution

The 1° retentate, collected from UF lines in the factory before the trials started, was pumped into a 2300 L balance tank, diluted with deionized water to 1% protein solution and pH was adjusted to 7.0 with 50% NaOH. The solution was pumped into the preheating circuit, consisting of a plate heat exchange and holding tubes, where it was heated to 80°C by steam and held for 20 min. After heating, the whey protein solution was cooled in cooling tubes by chilled water to 50°C and then pumped into the UF line. In the control run where no heat treatment was required, the 1° retentate was diluted (1% protein), adjusted pH to 7.0 and then pumped into UF plant.

3.2.6.2. UF/DF

The 1% whey protein solution from 1° retentate was pumped into UF plant. The UF plant consisted of four loops in series, where recirculation occurred around all loops (Figure 3.4). All loops had intercoolers before the booster pumps to maintain the temperature as required (47°C). DF demonised water was added at ~ 15%, ~ 45% and ~ 60% of initial solution volume after loop 1 and 2, 3 and 4, respectively. The membrane used was ICI polythersulfone (Niro Ltd., New Zealand). The retentate valve was opened when the total solid reached 23% and the 2° retentate was transferred to the spray drier.

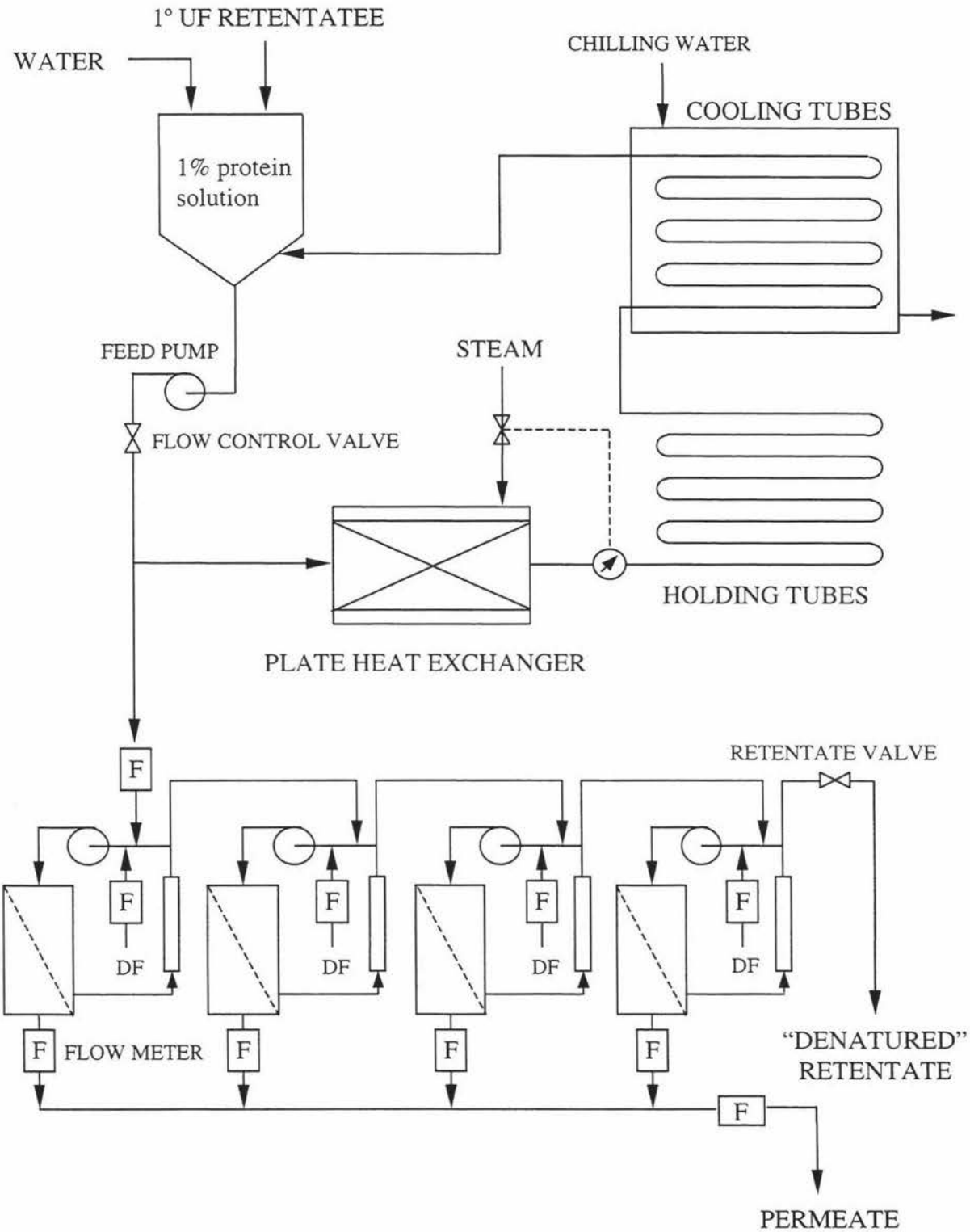


Figure 3.4. Procedure for retentate heat treatment and scheme of UF plant at Anchor Products

3.2.6.3. Spray drying

All WPC powders were manufactured using an atomizing Niro Production Minor Pilot Plant Drier (Niro, Copenhagen, Denmark) at Casein Plant, Edgecumbe. The drier, consisting of a drying chamber and a bag house (the cyclone by the drier), runs in multistage drying (MSD) mode. The inlet and outlet air temperatures used were 225°C and 80°C, respectively.

3.2.7. Preparation of WPC solutions

WPC solutions (5%, 10%, 12% and 15%, w/v, protein basis) were prepared by dissolving appropriate quantities of WPC powders in Milli-Q water followed by stirring for 2 hrs at room temperature using a magnetic stirrer, while degassing under vacuum to remove all visible air bubbles. The solutions were then adjusted to pH 7.0 with 1 N NaOH.

3.2.8. Preparation of viscous solutions and gels

The WPC solutions at protein concentrations of 5-15% were incubated at 5°C, 20°C or 40°C for 1 h, 5 h or 24 h (Figure 3.5). The viscosity or gel strength was then measured (Section 3.2.9 & 3.2.10)

3.2.8.1. Addition of Glucono-δ-lactone or salts

In these experiments, the final protein concentration in the solutions and gels was kept constant at 10%. Final concentrations of $CaCl_2$, NaCl and GDL added to the denatured or unheated WPC solutions were varied from 10 to 120 mM, 20 to 400 mM and 0.5 to 2.0% (w/w), respectively.

90 ml of 11.1% denatured or unheated WPC solution was mixed with 10 ml of $CaCl_2$ or NaCl solution at different concentrations and the pH was readjusted to pH 7.0 using 2N NaOH or HCl as required. In the case of GDL, WPC solution was mixed with 8-9.5 ml of water and then 0.5-2 g of GDL power was added. WPC and salt solutions were equilibrated to corresponding temperatures before mixing. Mixed solutions were transferred to plastic tubes as described above, or poured into small beakers (20 ml), covered with Parafilm and incubated at 5°C, 20°C, 30C or 40°C for ~ 4 hrs or 24 hrs.

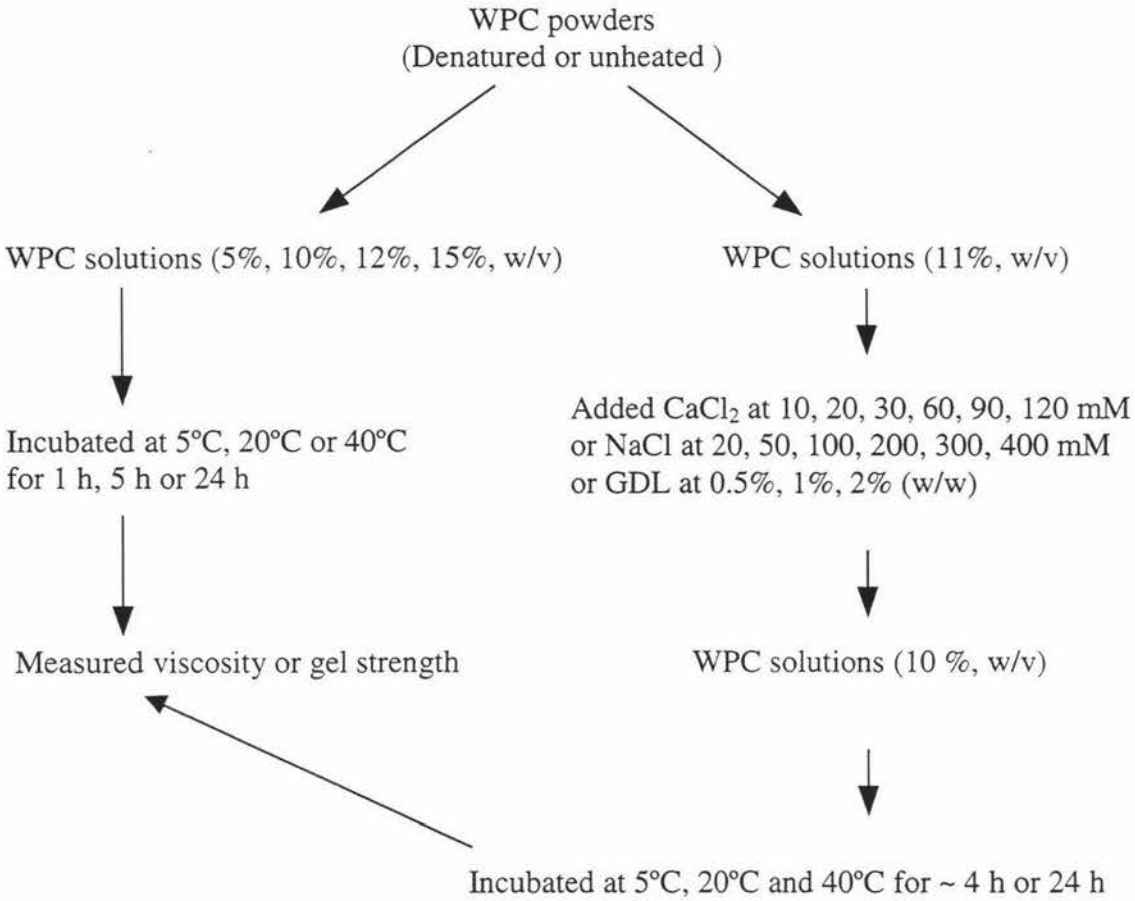


Figure 3.5. Preparation of viscous solutions or gels of WPC at different incubation temperatures.

3.2.8.2. Re-heating

Aliquots (20 ml) of 10% or 12% denatured or unheated WPC solutions were placed in plastic tubes (20 mm i.d. × 300 mm) and sealed at one end. The plastic tubes were then tightly tied at the other end using a rubber band and placed in a water bath that was thermostatically controlled at 50°C, 60°C, 70°C, 80°C or 95°C. The tubes were removed after 30 min or 60 min, placed immediately in ice water for 30 min and kept at 4°C overnight.

3.2.9. Measurement of viscosity

The viscosities of denatured or undenatured WPC solutions in the presence and absence of GDL and salts were measured by rotational viscometer system RHEOLAB MC 1 (Physica Meβtechnik GmbH, Stuttgart, Germany). The concentric cylinders, consisting of a 12.5 mm radius rotating bob and a 13.56 mm radius stationary cup, was used as measuring system in all experiments. All measurements were made with 17 ml of WPC solution, which was incubated for a given time at 5°C, 20°C or 40°C. The system temperature was controlled by a F25 Julabo Circulator (Julabo Labortechnik GMBH, Germany). Rotor rotation was started at shear rate of 1.291 sec^{-1} (1 rpm) and raised to 129.1 sec^{-1} (100 rpm) or 800 rpm in a stepwise manner. Shear rate then lowered from 129.1 (100 rpm) or 800 rpm in a stepwise manner. For each sample, solution was kept 5 min in the cup before rotation for temperature equilibration. Measurements of viscosity were logged to a PC, which interfaced to RHEOLAB MC 1 through an RS-232 serial port. The PC ran the data acquisition programme RheoSOLVE to control the shear rate and environmental conditions under which the viscosity was measured.

3.2.10. Compression test

Gels of WPC solutions formed by re-heating or addition of salts or GDL were tested gel strength using Instron (Model No. 4502, S. No. H3096). The external tubing was cut by a razor blade and then removed. Gel sections of 20 mm long were cut from the tubes using razor-edge cutting device. Compression test was performed on the 15 mm i.d. × 20 mm cylindrical gel samples by vertically compressing them with flat plates at the crosshead speed of 60 mm/min for 50% of their original height using 10 N head load. The force at the maximum displacement or the first breaking point was taken as a measure of gel strength for each gel.

CHAPTER 4
COMPARISON OF AGGREGATION BEHAVIOUR OF WHEY PROTEINS IN ACID WHEY AND ULTRAFILTRATION RETENTATE

4.1. Introduction

Whey protein concentrates (WPCs) are widely used as food ingredients because of their high nutritional value and desirable functional properties, such as heat-induced gelation. However, there is a great variation in the functional properties of WPC which may result from compositional differences due to the method of production and source of the whey (de Wit *et al.*, 1988). A lot of research currently being undertaken worldwide to improve functional consistency of WPCs and to diversify their functional application in the food industry. One of these potential applications is cold gelling WPC. This includes production of pre-denatured WPC which is capable of forming gels with minimal or without heat treatment. The degree of denaturation and aggregation of whey proteins in the pre-denatured WPC is considered to have significant effect on the functional properties of WPC.

Studies on the thermal properties of whey proteins have indicated that denaturation level as well as aggregates formed during heat treatment vary strongly with experimental heating conditions (McSwiney *et al.*, 1994a,b; Nielsen *et al.*, 1996; Gezimati, *et al.*, 1997; Havea, 1998; Havea *et al.*, 1998; Ju & Kilara, 1998b). Small "soluble" aggregates formed during heat treatment are considered to be responsible for forming a "fine-stranded" gel with good water holding capacity (Langton & Hermansson, 1991; Doi, 1993; Barbut, 1995). To achieve the desired functionality of WPC, it is necessary to determine the appropriate heating conditions (protein concentration, pH, ionic strength, heating temperature and holding time) for the formation of specific "soluble" protein aggregates.

As discussed in Section 2.7.4, salt type and concentration have a significant effect on the heat-induced denaturation and aggregation of whey proteins. To control the aggregate size during protein denaturation, it is desirable to have a low ionic environment (Parris *et al.*, 1993; Ju & Kilara, 1998b; Verheul *et al.*, 1998). Therefore, UF retentate obtained from acid WPC processing was chosen as the experimental sample because it has a lower ionic concentration (calcium, potassium and sodium) compared to that of the original whey. Previous researches demonstrated that thermal

denaturation and aggregation of whey proteins were pH-dependent due to intra-molecular interactions (Donovan & Mulvihill, 1987; McSwiney *et al.*, 1994a).

In this study, a range of preliminary experiments was carried out to investigate the effects of heat treatment, protein concentration and pH on denaturation and aggregation of whey proteins in the whey system to determine appropriate heating conditions for producing fine "soluble" whey protein aggregates. The 1° UF retentate were diluted to give protein concentration in the range of 1-10% and adjusted to pH 7.0 or 7.5. The solutions were then heated at 80°C or 95°C for various times as described in Section 3.2.3. Denaturation and aggregation of whey proteins during heating were determined using PAGE. Similar experiments were carried out on acid whey used in the manufacture of UF retentate for comparison.

4.2. Compositional analyses

The results of compositional analyses of acid whey, 1° UF retentate and 1% whey protein solution (prepared by diluting the 1° retentate with water) are listed in Table 4.1. The 1° retentate contained 19.83% total protein, while the protein content of acid whey was only 0.76%. The ash content of acid whey was 42-fold higher than that of 1% whey protein solution and approximately twice that the UF retentate, indicating that acid whey had much higher concentration of mineral, hence higher ionic strength than the UF retentate. The pH value of acid whey and UF retentate was ~ 4.3.

Table 4.1. Composition of whey protein solutions

Component (%)	Acid whey	UF Retentate	1% protein solution
Protein (N × 6.38)	0.76 ± 0.01	19.83 ± 0.07	1.00 ± 0.07
Ash	0.84 ± 0.04	0.44 ± 0.01	0.02 ± 0.01
Total solids	5.32 ± 0.01	24.35 ± 0.03	1.23 ± 0.03

4.3. Characteristics of heated whey protein samples

4.3.1. Native-PAGE pattern of heated UF retentate solutions

Under non-dissociating native conditions, the mobility of proteins is primarily determined by the charge-to-mass ratio on the protein molecule. In the typical native-PAGE of unheated whey proteins, β-Lg appeared as two bands in the order of β-Lg A and β-Lg B, which migrated faster than α-La and BSA. BSA was the slowest among the major whey proteins.

The typical native-PAGE patterns of heated (80°C or 95°C) whey protein solutions (1% protein, pH 7.0 or pH 7.5) prepared from 1° UF retentate are shown in Figure 4.2. The band intensities of monomeric whey proteins decreased gradually with heating time at 80°C and a little more rapidly at 95°C. The band of BSA disappeared after heating beyond 10 and 5 min, respectively, at 80°C and 95°C. Several bands appeared during heating in the region between α-La and the start of the stacking gel. These bands represented intermediate molecular weight aggregate species (Havea, 1998) and were not found in the unheated samples (Figure 4.2, left lanes). There was also high molecular weight material (labelled as large aggregate) caught on the top of stacking and resolving gels or within the stacking gel. The decrease in band intensities of native proteins with heating time was accompanied by concomitant increase in band intensities of intermediate aggregates and high molecular weight materials both at 80°C and 95°C. This material probably contained high molecular weight protein aggregates cross-linked via non-covalent interactions and disulphide bonds as suggested by McSwiney *et al.* (1994) and Havea *et al.* (1998).

At 80°C, two faint bands appeared between α-La and BSA. After heating beyond 5 min, one of the bands became denser, which was labelled as dimer. Another band with lower mobility than this dimer appeared in this region. This band probably was trimer. There were three diffuse bands in the region between BSA and the start of resolving gel after heating 10 min. Among them, the band just behind BSA, presumably tetramer, became apparent with heating time. The other two bands behind BSA might be BSA-β-Lg or BSA-α-La complex. The band intensities of all these intermediate aggregates increased with heating time, suggesting protein aggregates of different sizes were formed during heat treatment.

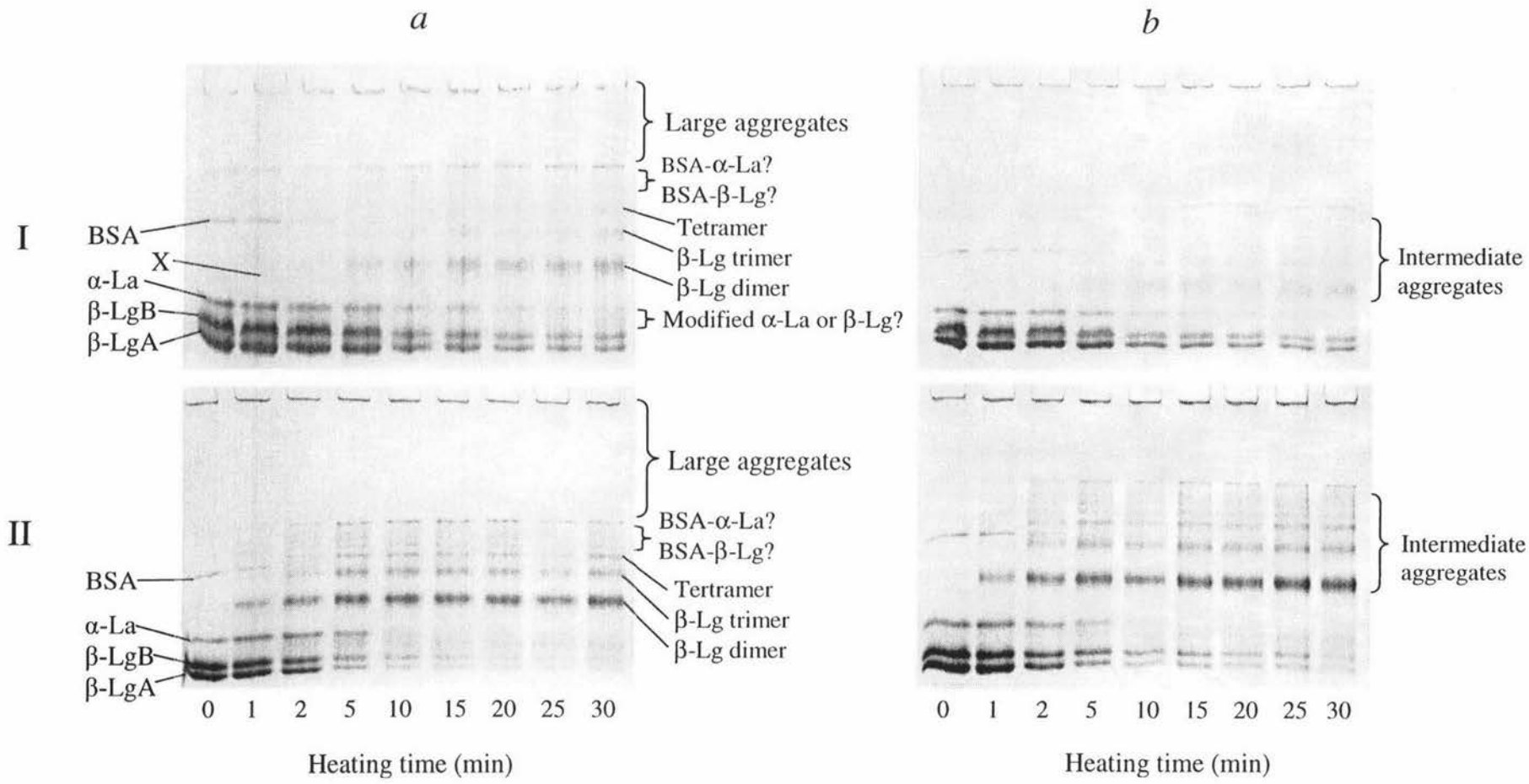


Figure 4.2. Typical native-PAGE patterns of retentate solutions, pH 7.0 (*a*) and pH 7.5(*b*) heated at 80°C (I) and 95°C (II) for 0, 1, 2, 5, 10, 15, 20, 25, and 30 min.

At 95°C, the appearance of these intermediate aggregate bands occurred much earlier, after heating 1 min. The bands, probably BSA-β-Lg or BSA-α-La complex, observed in the region of between BSA and the start of resolving gel were more obvious at 95°C than at 80°C. There were more high molecular weight aggregates formed at 95°C than at 80°C as expected (Barbut & Foegeding, 1993; Ju & Kilara, 1998b).

Similar native-PAGE patterns can be observed for 1% whey protein solutions at pH 7.0 and pH 7.5 (Figure 4.2) heated either at 80°C or at 95°C. Decreasing band intensity of native whey proteins with heating time was almost balanced by an increase in band intensity of intermediate aggregate species and an accumulation of high molecular weight aggregates on the top of stacking and resolving gels.

4.3.2. Loss of proteins during heating

The native-PAGE results of the heated whey protein solutions were quantified using the method described in Section 3.2.4. The changes in the intensity of protein bands were determined by densitometric scanning of the gels. The typical changes of band intensity (the peak area) corresponding to different proteins after heat treatment are shown in Figure 4.3.

The intensity (peak area) of each protein band in the heated whey protein solutions was reported as a percentage of the corresponding band in the unheated control sample. Figure 4.4 shows the quantitative results of native-PAGE when 1% whey protein solutions (pH 7.0) prepared from 1° retentate were heated at 80°C up to ~ 60 min. The results showed that the loss of native whey proteins, β-Lg, α-La and BSA increased with heating time. The loss of whey proteins was fast at the beginning of heating and slower as heating progressed. Heating beyond 20 min caused only minor changes in protein denaturation. This was probably because the aggregates formed during heating functioned as inhibitor for the reaction, i.e. hindered the free movement of active proteins (van Boekel, 1996).

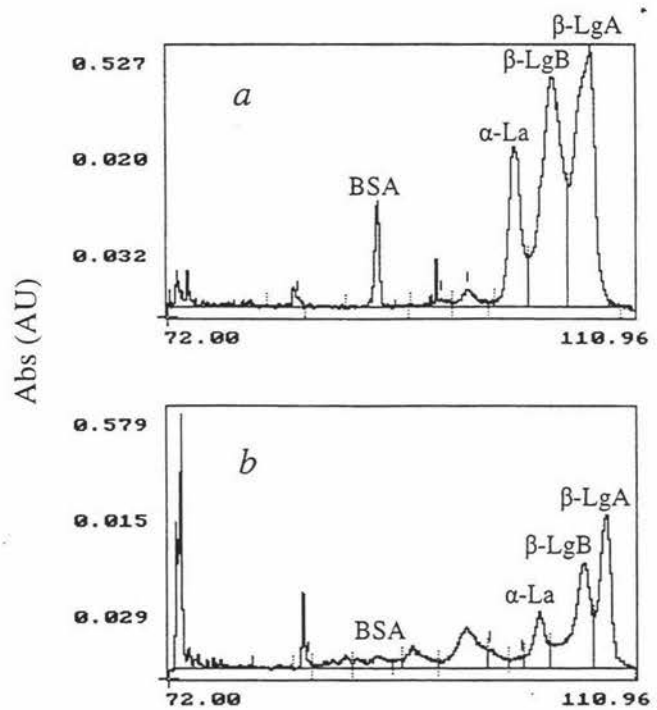


Figure 4.3. Changes in the band intensity of whey proteins in 1% protein solution from retentate between without heating (*a*) and heating at 80°C for 10 min (*b*).

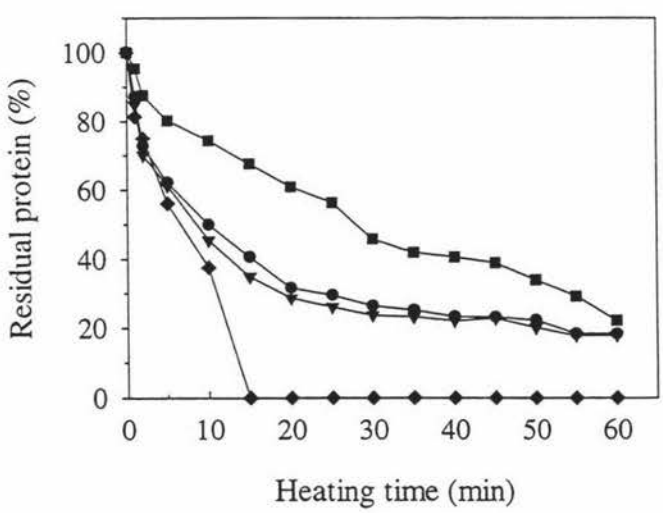


Figure 4.4. Loss of β-Lg A (●), β-Lg B (▼), α-La (■) and BSA (◆) in 1% whey protein solutions prepared from 1° UF retentate heated at 80°C.

BSA denatured more extensively than β-Lg and α-La, and the loss of β-Lg was greater than that of α-La, supporting previous findings that α-La was most resistant to heat denaturation (Dannenberg & Kessler, 1988; Singh & Creamer, 1991; Oldfield *et al.*, 1998). However, the observation that α-La denatured slower than β-Lg was contrary to the results of Hollar *et al.* (1995) and Havea *et al.* (1998), who investigated the thermal denaturation of α-La and β-Lg in 16% (total solid) sweet WPC and 12% WPC at 71°C and 75°C, respectively. The differences are due to many factors, such as the differences in composition, protein concentration, and heat treatment.

The extents of denaturation of the two genetic variants of β-Lg were similar. However, β-Lg A tended to be slightly more stable to heat treatment than β-Lg B. The result was in line with that of Nielsen *et al.* (1996) who found that denaturation of β-Lg was concentration dependence. β-Lg B denatured faster than β-Lg A at low protein concentration (1.5% or 2.5%), whereas β-Lg A was more sensitive to heat treatment at high concentration (10% or 15%).

Quantitative results of native-PAGE (Figure 4.2) are shown in Figure 4.5. Each point is the average result of duplicate experiments and the error bars of some points represent the typical standard deviation. Loss of β-Lg A, β-Lg B, α-La and BSA from 1% protein solutions increased with heating time at both 80°C and 95°. The resistance of major whey proteins to heat denaturation followed the order: α-La > β-Lg A > β-Lg B > BSA. In comparison, the loss of whey proteins occurred more rapidly at 95°C than that at 80°C. There was no significant difference on rates of protein denaturation when 1% whey protein solutions were heated at either pH 7.0 or pH 7.5. However, the loss of β-Lg A, β-Lg B and α-La, in general, were slightly greater at pH 7.5 than that at pH 7.0.

4.3.3. Heated acid whey

The native-PAGE patterns of acid whey (Figure 4.6) revealed that the intensity of major whey protein bands decreased with heating time. The decrease in band intensity of native proteins was much more rapid for samples heated at 95°C than those at 80°C, and both decreased faster than that in the heated 1% whey protein solution from 1° retentate (Figure 4.2).

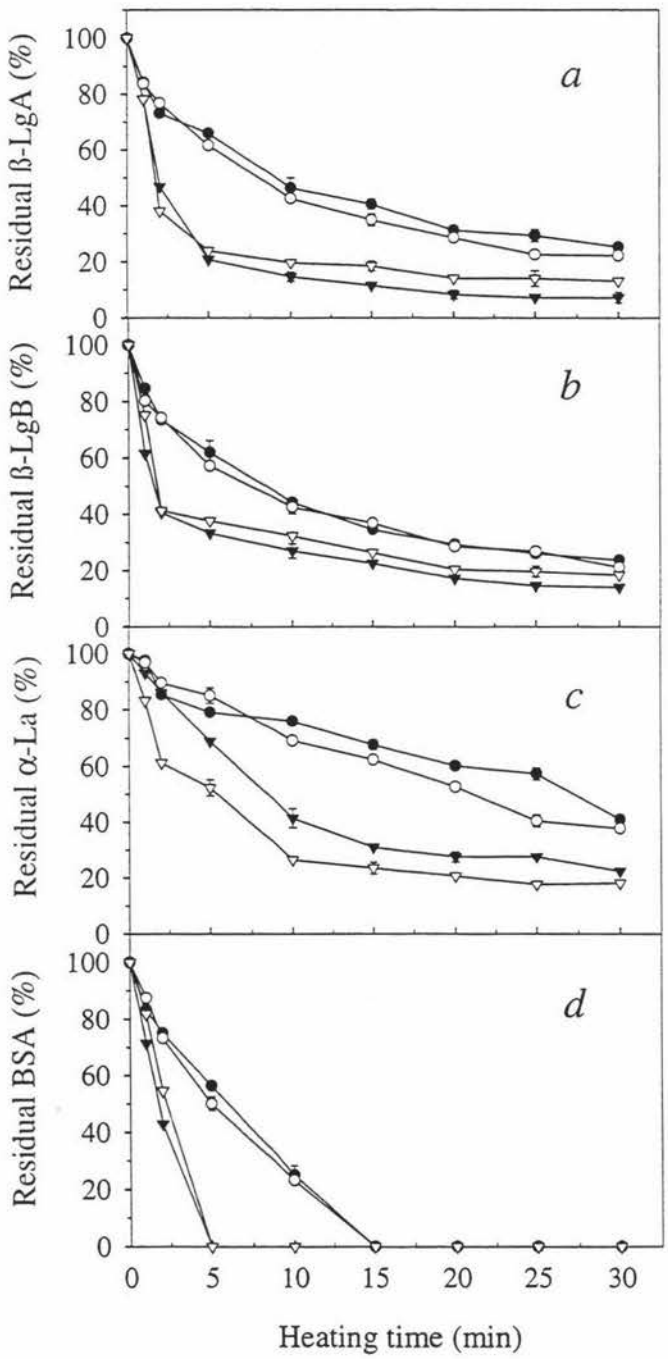


Figure 4.5. Loss of β-Lg A (*a*), β-Lg B (*b*), α-La (*c*) and B-SA (*d*) from retentate solutions (1% protein), pH 7.0 (● ▼) and pH 7.5 (○ ▽), heated at 80°C (● ○) and 95°C (▼ ▽).

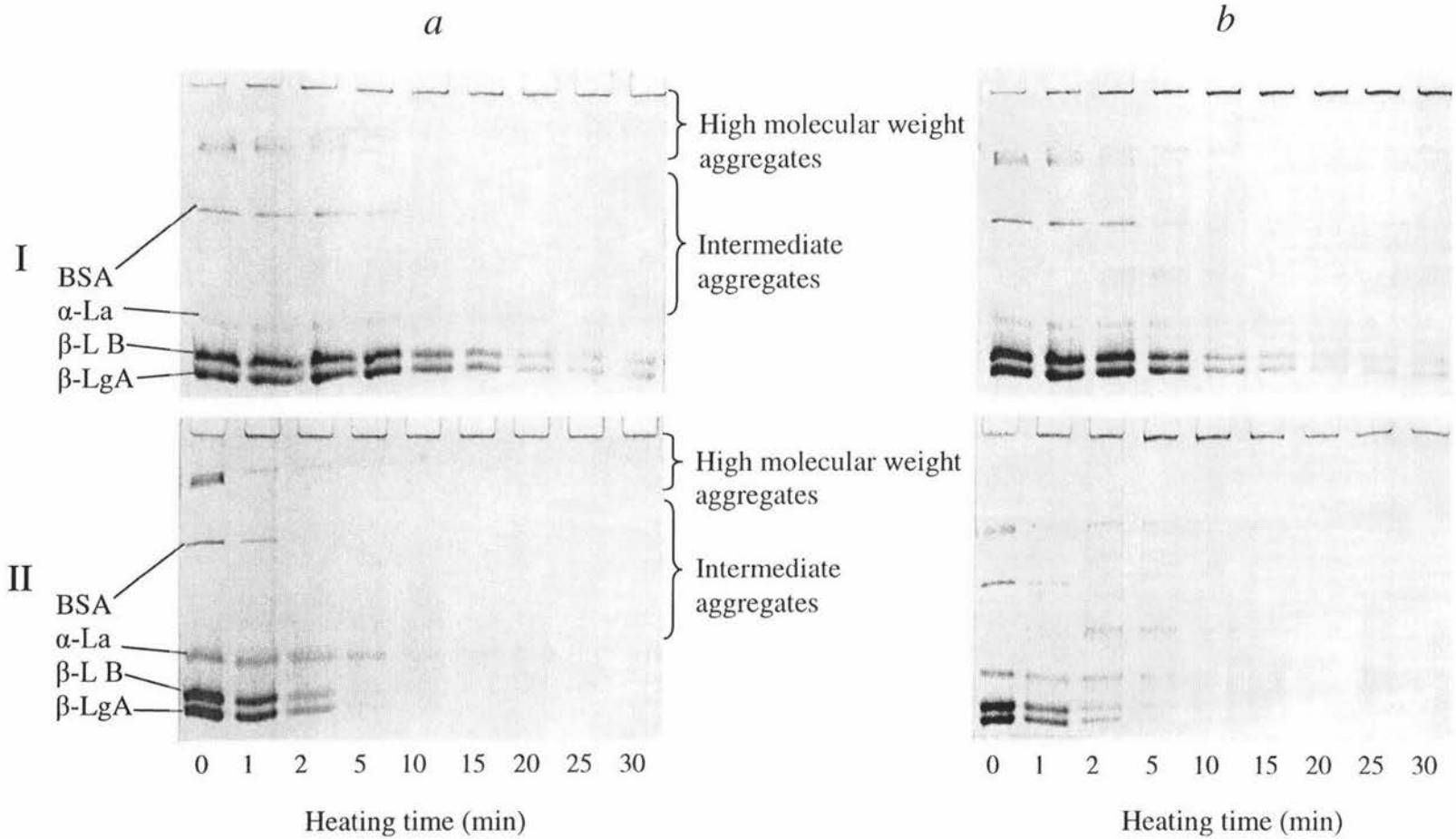


Figure 4.6. Typical native-PAGE patterns of acid whey, pH 7.0 (*a*), pH 7.5(*b*) heated at 80°C (I) and 95°C (II) for 0, 1, 2, 5, 10, 15, 20, 25, and 30 min.

The other major difference in native-PAGE patterns between the heated acid whey and retentate solution was that less low molecular weight intermediate aggregate species were formed in acid whey than that in retentate solution during heating at 80°C and 95°C. However, there was little high molecular weight material caught on the top of stacking and resolving gels in heated acid whey. It was clear that when the retentate solutions were heated, the loss of native whey proteins gave rise to predominantly low molecular weight intermediate aggregates (Figure 4.2). By contrast, when samples of whey were heated, very little intermediate aggregates were formed (Figure 4.6).

The different PAGE patterns obtained for heated acid whey and retentate solutions could be explained as follows. The whey protein aggregates formed during heating had to be within a specific size range in order to be caught on the top of stacking and resolving gels (Havea, 1998). If the protein aggregate was larger than the pore of the stacking gel, it would have stayed on the top of the stacking gel and would have been washed away during staining or destaining of the gels. It was possible that large aggregates formed from whey were larger than the pore size of the stacking gel and was not visible in the PAGE pattern.

When comparing the native-PAGE patterns of acid whey heated at pH 7.0 with that at pH 7.5, a slight difference was observed (Figure 4.6). Less intermediate aggregates formed in heated acid whey at pH 7.0 than that at pH 7.5. The latter also appeared to have a larger accumulation of high molecular weight aggregates caught on the top of stacking gel, suggesting that higher pH lead to the formation of larger aggregates. The observation of the native-PAGE patterns (Figure 4.6) was confirmed by the densitometric quantitation that the major whey proteins in acid whey heated at pH 7.5 were denatured faster than that at pH 7.0 (Figure 4.7).

The quantitative results of native-PAGE patterns showed that after heating less native proteins remained in acid whey than that in retentate solution (Figure 4.7). The different extent of denaturation between heated whey and retentate solutions could be attributed to the fact that they have different ionic environments. Acid whey had much higher concentrations of Ca^{2+}, Mg^{2+} and Na than did the retentate solution. These minerals promote the denaturation and aggregation of whey proteins on the alkaline side of the isoelectric point (Varunsation *et al.*, 1983).

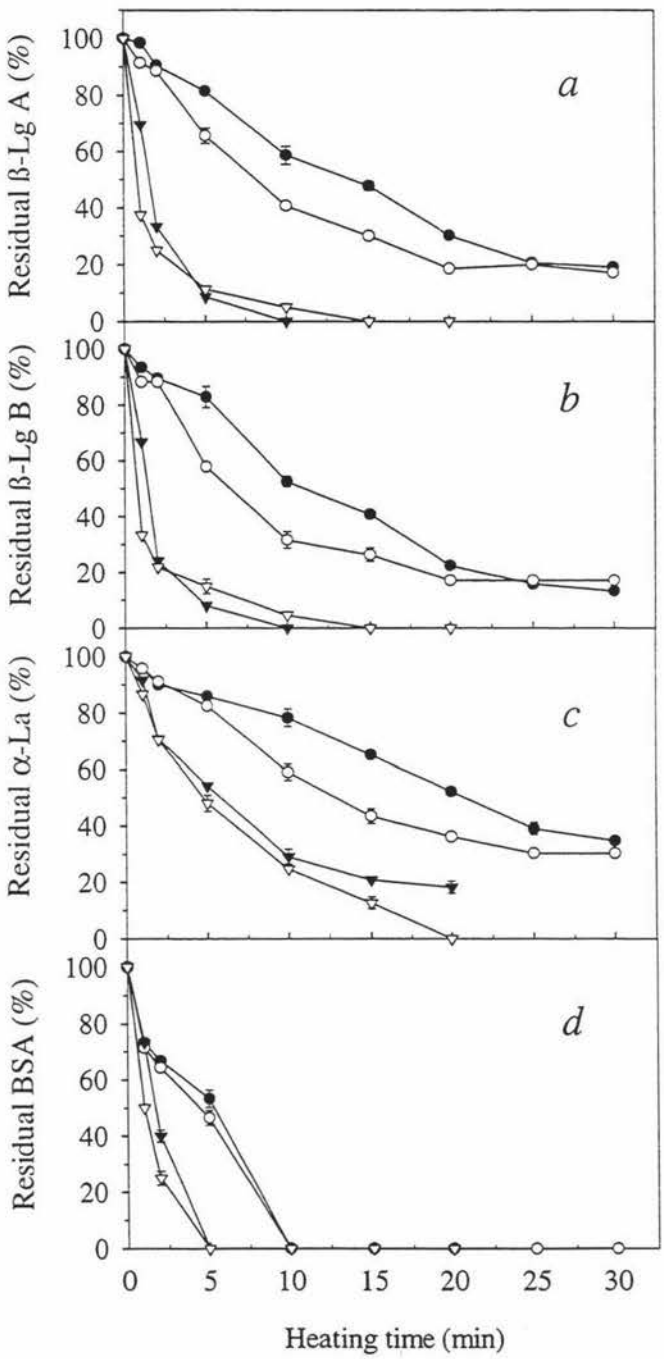


Figure 4.7. Loss of β-Lg A (*a*), β-Lg B (*b*), α-La (*c*) and BSA (*d*) from whey, pH 7.0 (● ▼) or pH 7.5 (○ ▽), heated at 80°C (● ○) or 95°C (▼ ▽).

4.4. Effect of protein concentration on whey protein denaturation in UF retentate

UF retentate was diluted to give 1-10% protein solutions and adjusted pH to 7.0 prior to heating (80°C, ~ 10 min). The native-PAGE patterns of heated retentate solutions with different protein concentrations are presented in Figure 4.8. The samples of 10% whey protein solution heated at 80°C for 5 and 10 min were not analysed since they formed gels. The native-PAGE patterns of heated 1% and 2% protein solutions were similar to that of 1% protein solution (pH 7.0, 80°C) in Figure 4.2. There were three bands in the region between BSA and α-La. The intensity of the two bands labelled as dimer and trimer increased with heating time (Figure 4.8*a*, *b*). In contrast, dimer and trimer were faint and decreased with heating time in the native-PAGE of 5% and 10% protein solutions (Figure 4.8*c*, *d*). It indicated that intermediate molecular weight protein aggregates were mainly formed during heating from solutions of low protein concentrations compared to that of high protein concentrations. Furthermore, there were several faint bands behind BSA which appeared to be clearer in 1% whey protein solutions than that in 2% protein solutions heated for 5 and 10 min but not in 5% protein solutions. These bands probably corresponded to higher molecular aggregate species (e.g. β-Lg tetramer). The results suggested that the formation of various intermediate protein species (dimers, trimers and tetramers etc.) were more prominent in the retentate solution with lower protein concentrations i.e. in the order of 1% > 2% > 5% > 10%.

There was an accumulation of large aggregates that failed to migrate into the resolving gel. These aggregates increased with protein concentration and initially can be clearly observed in 2% protein solution heated for 5 min (Figure 4.8*b*). The accumulation of large aggregates was more obvious in heated 5% and 10% protein solutions than that in heated 1% and 2% protein solutions. The result was in good agreement with those of Nielsen *et al.* (1996) and Havea *et al.* (1998), who found the extent of the aggregation appeared to increase with protein concentration in β-Lg and WPC solutions, respectively. Ju & Kilara (1998b) also demonstrated that both the extent and the size of aggregates increased with increasing whey protein concentration when 1-9% WPI solutions were heated at 80°C for 30 min.

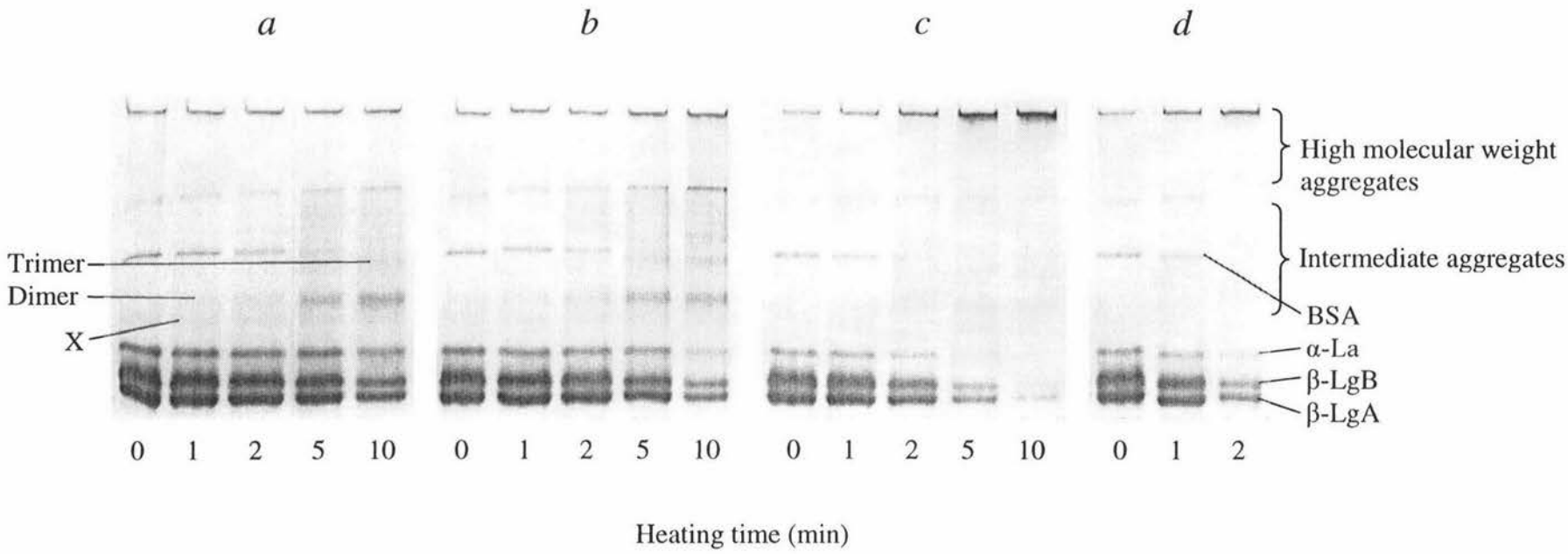


Figure 4.8. Native PAGE of 1% (*a*), 2% (*b*), 5% (*c*) and 10% (*d*) whey protein solutions (pH 7.0) from UF retentate heated at 80°C for 0, 1, 2, 5 and 10 min.

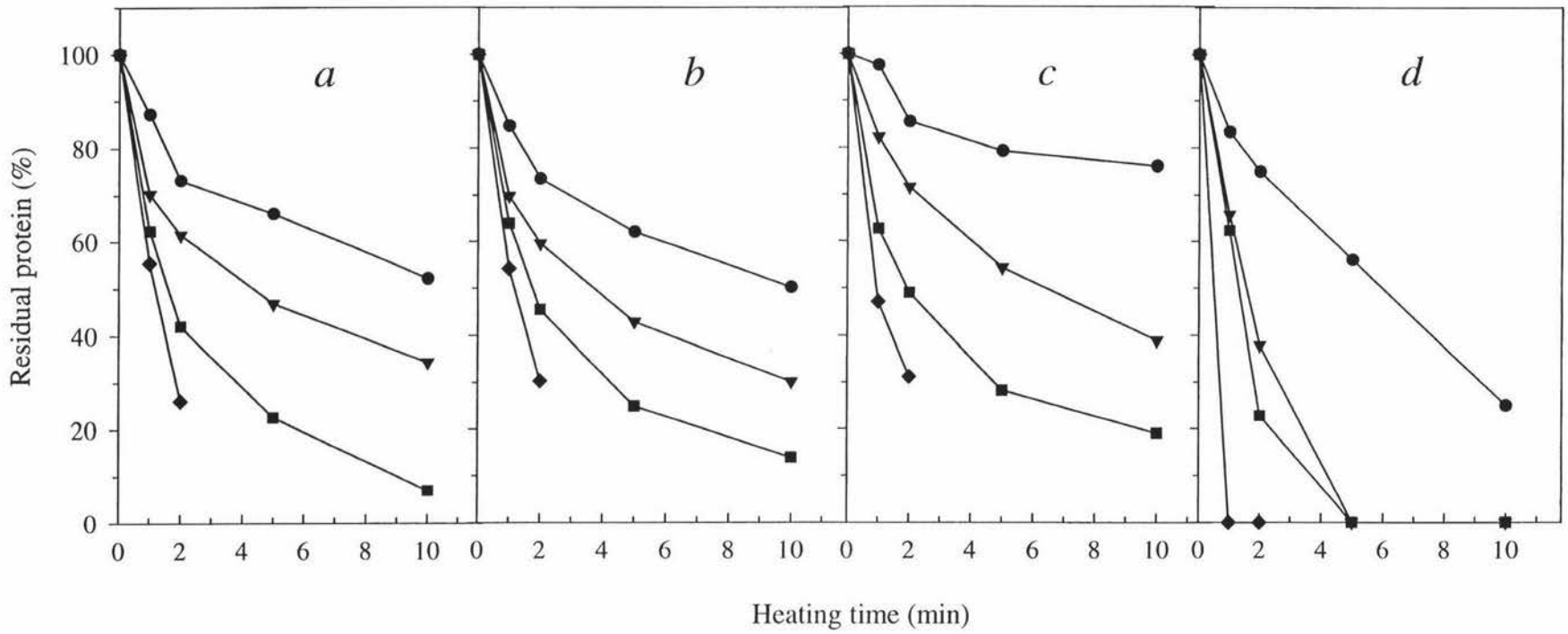


Figure 4.9. Loss of native β-Lg A (*a*),β-Lg B (*b*),α-La (*c*) and BSA (*d*) from retentate solutions (pH 7.0) containing 1% (●), 2% (▼), 5% (■) and 10% (◆) protein heated at 80°C.

Quantitative result of the native-PAGE patterns (Figure 4.8) is shown in Figure 4.9. The extent of loss of whey proteins with heating time at 80°C was greatest in 10% protein solution followed by 5%, 2% and 1% protein solutions.

4.5. Discussion

The purpose of this preliminary study was to establish the most suitable heating conditions (protein concentration, pH, ionic strength, heating temperature and time) for the manufacture of a denatured WPC product, capable of gel formation upon rehydration with minimal or no heat treatment. Earlier investigation (Havea *et al.*, 1998) showed that heating 1% WPC solution (pH 6.8) at 75°C resulted in formation of predominantly intermediate "soluble" aggregates (dimers, trimers etc.). By contrast, heat treatment of 10% WPC solutions under the same conditions gave rise to the formation of large "insoluble" aggregates. For a WPC product with desired functional properties (i.e. cold gelling), it is desirable to have predominantly soluble aggregates. The formation of such aggregates is a function of not only protein concentration, but also pH and ionic strength of the solution.

In this study, therefore, the effects of protein concentration, heating temperature and pH on denaturation and aggregation of whey proteins in acid whey and 1% whey protein solution prepared from 1° retentate were studied using PAGE. The results indicated that denaturation and aggregation behaviour of whey proteins were different in two systems. The loss of native protein was faster from heated whey than that from heated retentate solutions. The loss of native protein in heated retentate solution was accompanied by formation of predominantly intermediate aggregates and some high molecular weight aggregates, which were caught on the top of stacking and resolving gels (Figure 4.2). By contrast, the loss of native proteins in heated whey was accompanied by formation of minimal quantities of intermediate aggregates and high molecular weight aggregates (Figure 4.6). Some of the large "insoluble" aggregates appeared to have been lost from the system as the intensities of the aggregated materials did not appear to account for all the lost native proteins.

The differences between the aggregation behaviour of proteins in heated whey and diluted retentate were due largely to the different ionic environment between the two systems (see ash contents of whey and retentate, Table 4.1). In the acid whey, all

the minerals were present. Among these minerals, Ca and Mg were reported to facilitate the formation of large "insoluble" aggregates (Heava, 1998; Heava *et al.*, 2000). At pH 7.0 or 7.5, the whey proteins are negatively charged. The positively charged ions can bind easily to the proteins, resulting in reduction of the repulsive forces between the negatively charged proteins. The denatured proteins can come closer to each other forming hydrophobic and ionic bridges between them, resulting in the formation of large "insoluble" aggregates (Havea, 1998). This is the main reason for the observed faster loss of native proteins from heated whey (Figure 4.6 & 4.7).

In heated retentate solutions (1% protein, pH 7.0 or 7.5), much of the minerals have been removed during the UF process, so the ionic strength of the solution is relatively low. At pH 7.0 or 7.5, the negatively charged proteins and low ionic strength provided optimal conditions where there would be a good balance between the attractive and repulsive forces between the denatured protein molecules (Tang *et al*, 1995; Havea, 1998). Such conditions were suitable for formation of predominantly disulphide linked small aggregates, hence the observed intermediate aggregates in heated retentate solutions (Figure 4.2). The formation of these aggregates was a relatively slow reaction as shown in Figure 4.2 and 4.5. It was decided, therefore, that for the purpose of this study, it was more appropriate to heat the diluted retentate instead of acid whey.

It was necessary to choose the most appropriate protein concentration for heat treatment of retentate solutions. At higher concentration, > 2% protein, minimal intermediate aggregates were formed (Figure 4.8). It was therefore concluded that heating a lower concentration protein solution (i.e. 1% protein) was more suitable for the formation of the desired "soluble" intermediate aggregates. At higher protein concentration, the rate of aggregation was probably too fast that the soluble intermediate aggregates existed for a very short time before they were incorporated into insoluble aggregates (Figure 4.8*c*,*d*).

It was observed that at either 80°C or 95°C, up to 30 min, large quantities of soluble intermediate aggregates were formed. This is an important consideration because from an industry application point of view, there is certain degree of flexibility, i.e. if there is lack of control in the heating and cooling process, the product produced could still meet the designed functional properties because of the

soluble intermediate aggregates formed. For further investigation, it was decided to use a heating temperature of 80°C for 20 min.

The effect of pH (7.0 or 7.5) on the aggregation behaviour was not significant. (Figure 4.2). For further investigation, it was decided that heat treatment of diluted retentate solutions would be carried out at pH 7.0.

The results obtained in this preliminary study can be used as a guide for choice of a specific process for production of desired WPC products. Therefore, on the basis of the results from the present study, the possible conditions selected to apply in the pilot plant scale production of WPC would be adding a step of heat treatment (80°C for 20 min) to 1% retentate solution (pH 7.0). The heated retentate solution would be ultrafiltrated again to increase protein concentration prior to drying. It was considered that reasonable denaturation and aggregation of whey proteins would be obtained under such heating conditions, which could lead to a new WPC product with desirable functionality.

CHAPTER 5
PRODUCTION OF DENATURED WPC POWDERS AT MASSEY PILOT PLANT

5.1. Introduction

It has been generally accepted that the functional properties of WPC are affected by whey source and processing history which result in different composition and extent of denaturation (Morr & Foegeding, 1990). Besides the source of the whey, processing history, the technology used during manufacture is most important in contributing to the varied composition and denaturation of WPC. By applying specific processing conditions, the manufacture of WPC products could be modified to achieve the desired denaturation level to improve their functionality such as gelling properties. However, little information is available on the effect of degree whey protein denaturation on functionality of commercial WPC products.

The ability of whey proteins to form a gel at room temperature (i.e. cold gelation) is a new concept and has been considered to have potential applications in food industry (Barbut & Foegeding, 1993; Nakamura *et al.*, 1995). According to this procedure, an initial preheating step is required to denature whey proteins to form "soluble" aggregates. The addition of salts or acidulants is reported to cause formation of gels at ambient temperatures. However, studies carried out on cold-set gelation of whey proteins are limited to laboratory scale (Barbut & Foegeding, 1993; McClements & Keogh, 1995).

The effects of salt type and concentration have been the subjects of numerous investigations, primarily because of the strong influence of salts on gel characteristics. The effects of monovalent (Na, Li, K, Rb, Cs) and divalent (Ca, Mg, Ba) salts on gel structure are different (Kuhn & Foegeding, 1991). The presence of salts in heated WPC solutions alters the balance of attractive and repulsive forces between the denatured molecules, hence influencing the type of aggregates formed during heating. It is clear from the proceeding chapter that formation of the desired "soluble" intermediate aggregates requires low levels of mineral. Such aggregates can be isolated and dried as denatured WPC, which can form gels upon rehydration or addition of salts.

In the present study, an attempt was made to produce heat-denatured WPC powder, which would form viscous solution or gels upon re-hydration and addition of acidulant or salts. In the work carried out in Chapter 4, the process conditions for thermally dunaturing whey proteins were determined, i.e. 1% whey protein solution from retentate (pH 7.0) heated at 80°C for 20 min. A step of heat treatment using the determined heating conditions was added prior to UF in the current WPC manufacturing process, and WPC powders containing denatured whey proteins with "soluble" aggregates were produced in the pilot plant at Massey University. The extent of denaturation as well as the viscosity and gelation ability of WPC powders produced was determined.

5.2. Composition of WPC powders

The compositions of the WPC powders produced (Section 3.2.5) from Trial 1-4 are listed in Table 5.1. H0823 and H0913 were heat-denatured WPC, whereas U0823 and U0913 were produced by normal WPC process without preheating and were used as control.

Table 5.1. Composition of WPC powders produced at the Massey pilot plant

Trial No.	WPC powder	Protein (N × 6.38) (%)	Ash (%)	Total solids (%)
1	H0823	83.32 ± 0.42	2.42 ± 0.14	95.94 ± 0.06
2	H0913	82.60 ± 0.26	2.22 ± 0.29	95.25 ± 0.15
3	U0823	82.21 ± 0.33	2.27 ± 0.26	95.23 ± 0.31
4	U0913	81.27 ± 0.10	2.18 ± 0.15	95.16 ± 0.18

H = Heated; U = Unheated.

Protein levels of between 81% to 83% were consistently obtained for WPC powders made with or without preheating. The other components, ash, and total solids were also quite similar for all powders produced. pH values varied from 7.09 to 7.13.

5.3. Denaturation of whey proteins in heated WPC

Visually, the heat-denatured powders, H0823 and H0913 did not differ from that of unheated WPC, U0823 and U0923. However, the solutions of heat-denatured WPC powders were very viscous compared to that of unheated WPC solutions.

The native-PAGE analysis of the 1% (w/v) protein solution of WPC gave a good pictorial representation of the extent of whey protein denaturation (Figure 5.1). The intensities of whey protein bands of β-Lg A, β-Lg B, α-La and BSA in heat-denatured H0823 (lane 1) and H0913 (lane 2) were lower than that of the unheated U0823 (lane 3) and U0913 (lane 4). There was little intermediate aggregates appeared within the resolving gels in the heated WPC solutions, as was found in the heated retentate solution (Figure 4.2). The accumulation of high molecular weight aggregates on the top of stacking and resolving gels was more obvious in the heated WPC solutions than that in the unheated WPC solutions. The observation that larger aggregates formed in the WPC solution than did in the retentate solution under the same heating conditions suggested that different heating systems or drying might affect the aggregate size.

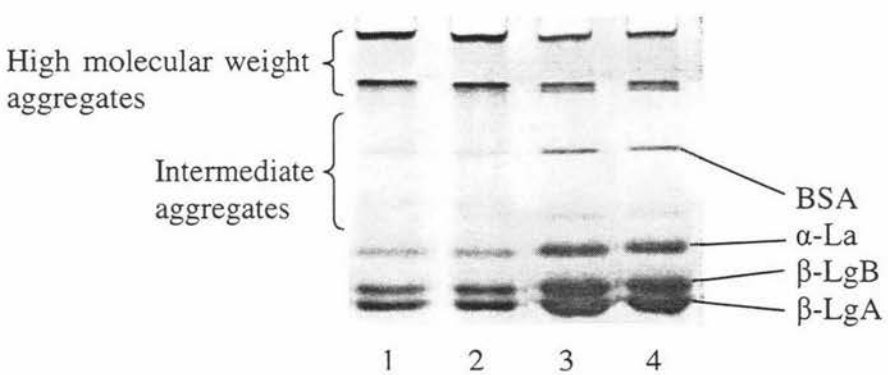


Figure 5.1. Native-PAGE patterns of the two heat-denatured WPC (lane 1, 2) and the unheated control WPC (lane 3, 4) solutions.

The extent of denaturation of whey proteins in denatured WPCs was estimated by quantitation of the native-PAGE. The observation of native-PAGE patterns was supported by the quantitative results (Table 5.2). The denaturation level of whey

proteins achieved was quite similar between the trials. Loss of β-Lg A and β-Lg B during 20 min heating at 80°C in H0823 and H0913 was similar.

Table 5.2. Denaturation of whey proteins in WPC powders (%)

Whey protein	H990823	H990913
β-Lg A	73.3 ± 1.4	73.2 ± 0.4
β-Lg B	75.4 ± 0.7	74.9 ± 0.8
α-La	73.0 ± 2.0	71.4 ± 1.5
BSA	80.9 ± 3.3	82.3 ± 0.9

5.4. Comparison of gelation properties of heat-denatured and unheated WPC

5.4.1. Effect of protein concentration and temperature

WPC solutions (10% or 12%, w/v) prepared (Section 3.2.7) from both the heat-denatured (H0823) and the control (U0823) WPCs were heated (Section 3.2.8) at 50-95°C for 30 or 60 min. The gelling properties of the WPC solutions (denatured and unheated) were determined.

At 12%, both denatured (H0823) and unheated control (U0823) WPC solutions formed self-supporting gels upon heating at ≥ 70°C, whereas at 10% they only formed very weak gels after heating at 95°C for 60 min. Weak gels were formed when both 12% WPC solutions were heated at 70°C for 30 min; these gels could not be tested.

Visually, the gels formed by heat-denatured WPC solutions were opaque, elastic and relatively weak, while the gels formed by unheated WPC solutions were clearer, brittle and firm. For both WPCs, the gel strength increased significantly with heating temperature and holding time at both concentrations, 10% and 12% (Figure 5.2). However, the strength of gels produced from the unheated WPC was greater than that from the denatured WPC.

Overall, the result of present study showed that gels formed by heat-denatured WPC solutions were not as strong as those formed by unheated WPC solutions, although the gels produced from denatured WPC appeared to be more elastic than that

of unheated WPC. It would be more interesting to investigate the cold-set gelling ability of the denatured WPC in comparison with the unheated WPC.

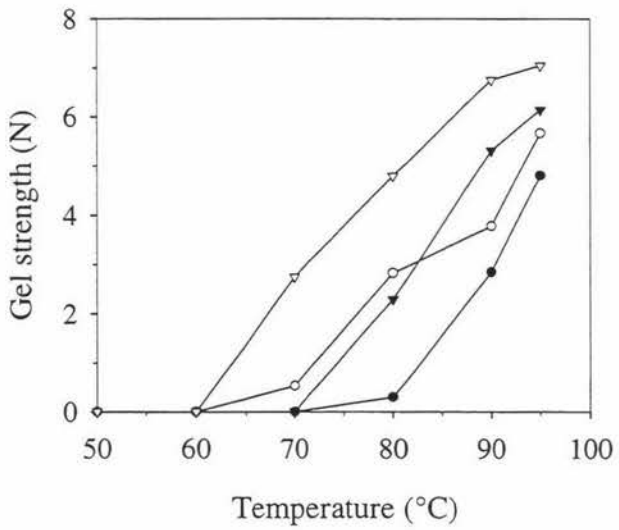


Figure 5.2. Gel strength of 12% heat-denatured (● ○) and unheated (▼ ▽) WPC solutions heated at various temperature for 30 (● ▼) or 60 (○ ▽) min.

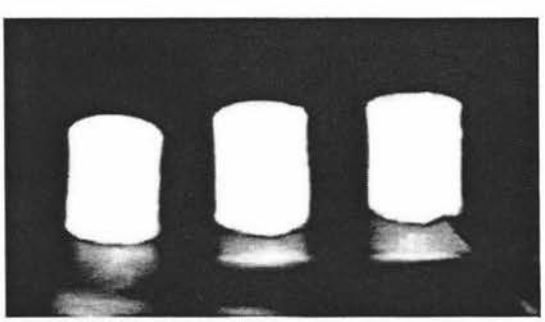
5.4.2. Effect of salts on gelation at low temperature

Different salts ($CaCl_2$ and NaCl) were added at different concentrations (Section 3.2.7) to induce gelation in 12% (w/v) protein solutions of heat-denatured WPC at 5°C, 20°C and 40°C for up to ~ 4 hrs. The 12% unheated WPC solutions were used as control.

Gels were formed by 12% denatured WPC (H0823) upon addition of $CaCl_2$ (15-360 mM) and incubation for 4 h at 40°C, but not at 5°C or 20°C. Low concentrations of $CaCl_2$ (5-10 mM) did not cause gelation of the denatured WPC. The minimum concentration of $CaCl_2$ required to cause gelation of 12% denatured WPC was 15 mM. This level was slightly higher than that reported by Hongsprabhas & Barbut (1997b) and Ju & Kilara (1998a), who used 5 and 10 mM, respectively, to induce gelation of 10% and 8% WPI solution incubated for 4 hrs at 45°C and dialysed

for 16 hrs at room temperature. This difference was probably due to the different mineral and protein concentrations in the systems used.

The gels formed by addition of $CaCl_2$ were translucent or opaque (Figure 5.3). Slight water separation (syneresis) was observed from the gels formed at higher $CaCl_2$ concentrations (≥ 120 mM). Gel opacity appeared to increase as $CaCl_2$ was increased from 15 to 360 mM, suggesting that larger aggregates were formed at high $CaCl_2$ concentrations. Barbut (1995) reported that increasing $CaCl_2$ concentration from 10 to 180 mM in 10% WPI suspensions progressively increased protein strand (aggregate) size as determined by scanning and transmission electron microscopy. The effects of $CaCl_2$ on the gel transparency can be partly explained by the DLVO theory (Friberg *et al.*, 1990). At low $CaCl_2$, in which gels are more translucent and have smaller aggregate size, the presence of a repulsive electrostatic energy barrier may slow down the aggregation of the pre-denatured protein molecules. This is in contrast to gels obtained at high $CaCl_2$, in which aggregation rate is much faster, mainly because of changes in the distance distribution of repulsive forces (Hongsprabhas & Barbut, 1996).



a *b* *c*

Figure 5.3. Photograph of gel cylinders from 12% heat-denatured WPC (H0823) upon addition of 30 (*a*) 240 (*b*) and 360 (*c*) mM $CaCl_2$ incubated at 40°C for 4 hrs.

The clear gels obtained at lower $CaCl_2$ had lower gel strength than that of the opaque gels obtained at higher $CaCl_2$. (Figure 5.4). Increasing $CaCl_2$ from 15 to 360 mM increased the gel strength. There was a sharp increase in gel strength with increase in $CaCl_2$ concentration up to ~ 30 mM and a plateau thereafter.

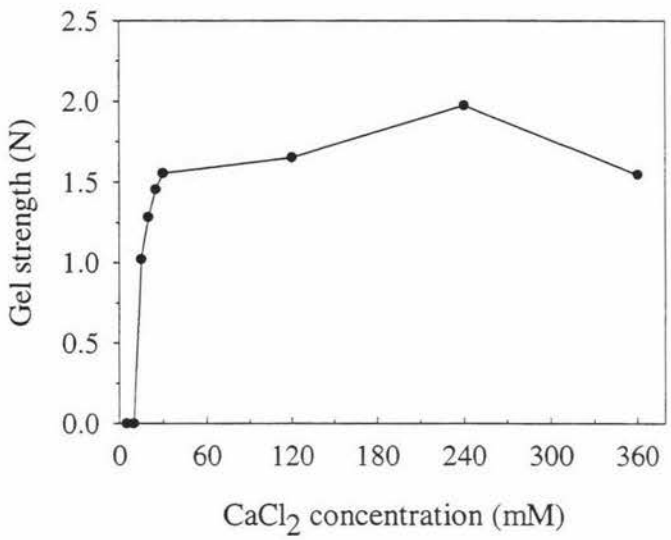


Figure 5.4. Effect of $CaCl_2$ addition on gel strength of 12% heat-denatured WPC (H0823) solution incubated at 40°C for 4 hrs.

To compare Ca-induced gelation of heat-denatured and unheated WPC, various concentrations of $CaCl_2$ were added to both 12% WPC solutions (heated or unheated) and incubated at 5-40°C. No gelation occurred in the unheated WPC solutions, confirming that the preheating step is essential for the cold-set gelation.

According to composition analysis and the extent of protein denaturation, little difference was found between the two heat-denatured WPC powders (see Table 5.1, 5.2). In Ca-induced gelation, similar trend of increase in gel strength with $CaCl_2$ concentration was observed in gels formed from H0823 and H0913 upon addition of 15-30 mM $CaCl_2$, indicating no trial difference in terms of Ca-induced gelation of denatured WPCs (Figure 5.5).

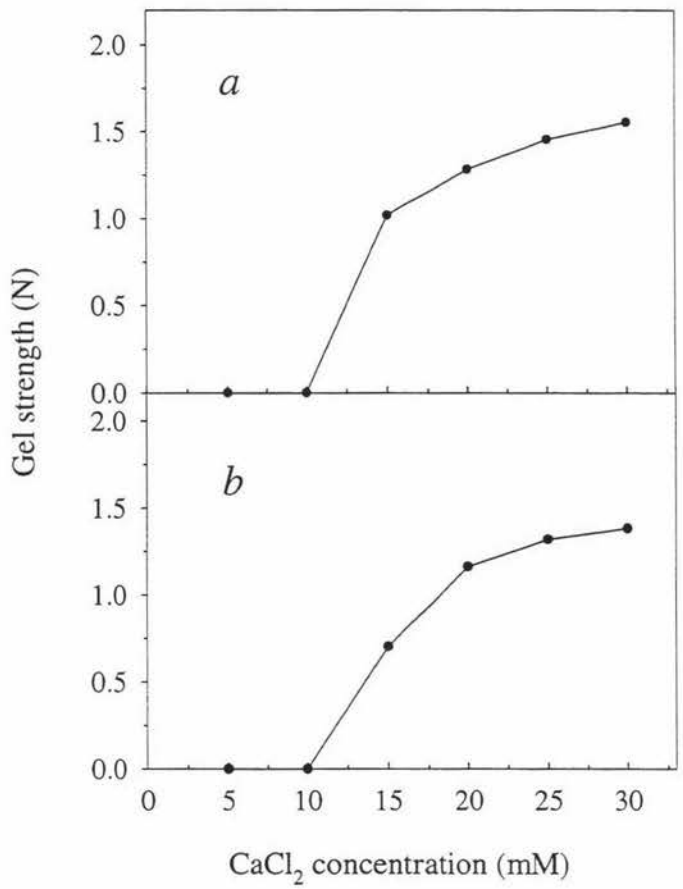


Figure 5.5. Comparison of gel strength as a function of $CaCl_2$ addition at 40°C in 12% heat-denatured WPC solutions prepared from H0823 (*a*) and H0913 (*b*).

In addition to Ca-induced gelation, an attempt was made to carry out Na-induced gelation of heat-denatured WPC. NaCl (50-400 mM) was added to 12% denatured or unheated WPC solutions at 5°C, 20°C and 40°C, respectively. However, no gel was formed from all samples after incubation for ~ 4 hrs. Kuhn & Foegeding (1991) showed different effects of mono-valent (Na, Li, K, Rb, Cs) and divalent (Ca, Mg, Ba) cation on gel formation of whey protein solutions. The minimum amount of concentration required to form a gel also differs between NaCl and $CaCl_2$, as $CaCl_2$ appeared to be more effective than NaCl (Mulvihill & Kinsella, 1988).

5.5. Viscosity of heat-denatured WPC

Heat-denatured or unheated WPC solutions (5%, 10% and 15%, protein basis) were prepared (Section 3.2.6) and incubated at 5°C, 20°C or 40°C for 1, 5 or 24 hrs. The viscosities of these solutions were measured over a wide range of shear rate (Section 3.2.9). Viscosity of the denatured WPC solutions, with a maximum of 43 Pa·s, was about 21 to 57-fold higher than that of the unheated WPC solutions depending on protein concentration and incubation conditions. Increased viscosity of denatured WPC solutions suggested the presence of whey protein aggregates in the solution.

The viscosity of denatured WPC solutions at different concentrations as related to shear rate at 5°C is shown in Figure 5.6. Similar patterns of viscosity were obtained for denatured WPC solutions incubated at 20°C or 40°C (Appendix 5.1 & 5.2). Generally the denatured WPC solution exhibited a thixotropic character, i.e. viscosity decreased with increasing shear rate. Viscosity of the denatured WPC solutions increased with protein concentration and incubation time at all shear rates tested (Figure 5.6). In contrast, the unheated WPC solutions had no viscosity changes upon incubation under any conditions.

In order to compare the combination effect of incubation temperature and time on the viscosity of denatured WPC solutions, the initially measured viscosity of 10% denatured WPC solutions at 15.15 sec^{-1} were used (Figure 5.7). At a given protein concentration and shear rate, the viscosity decreased with increasing in temperature and increased with increasing in time. The increase in viscosity with incubation time was more obvious at 5°C than that at 20°C or 40°C.

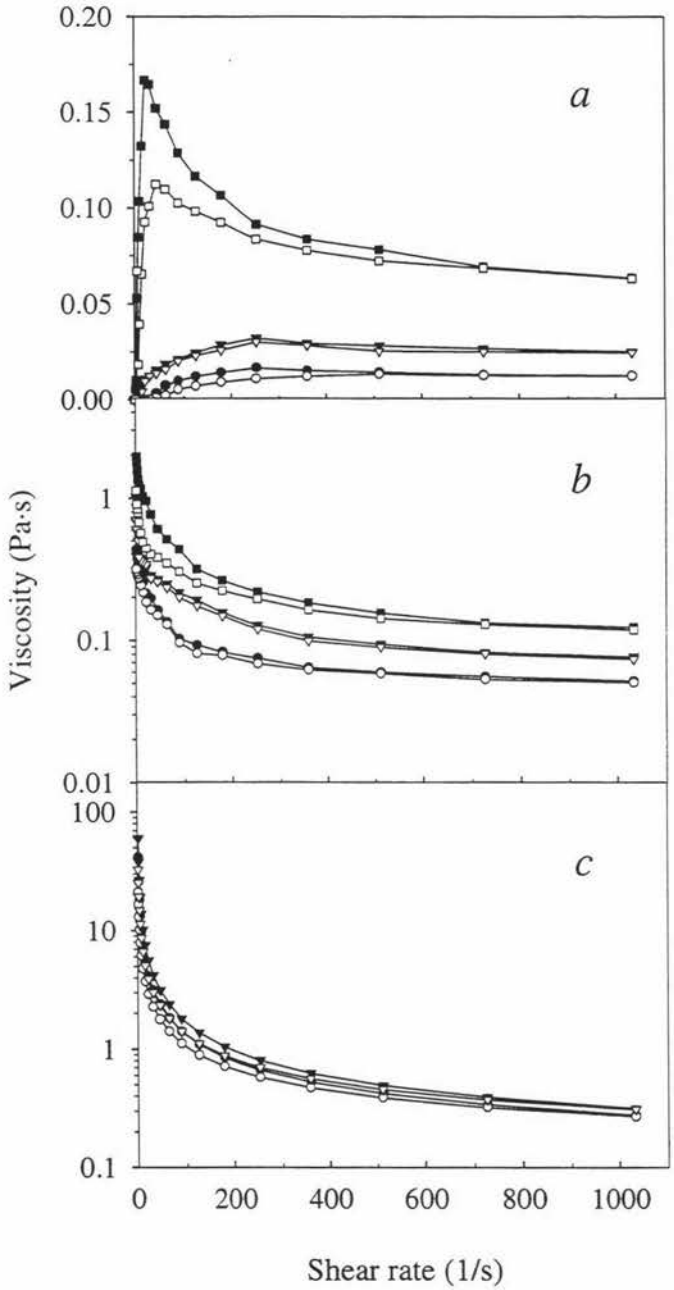


Figure 5.6. Viscosity of 5% (*a*), 10% (*b*) and 15% (*c*) heat-denatured WPC solutions (H0913) incubated at 5°C for 1 h (● ○), 5 h (▼ ▽) or 24 h (■ □) as a function of shear rate. Shear rate increased from 0 to 1032.5 sec^{-1} (800 rpm) (● ▼ ■) and then decreased from 1032.5 to 0 sec^{-1} (○ ▽ □) in a step manner.

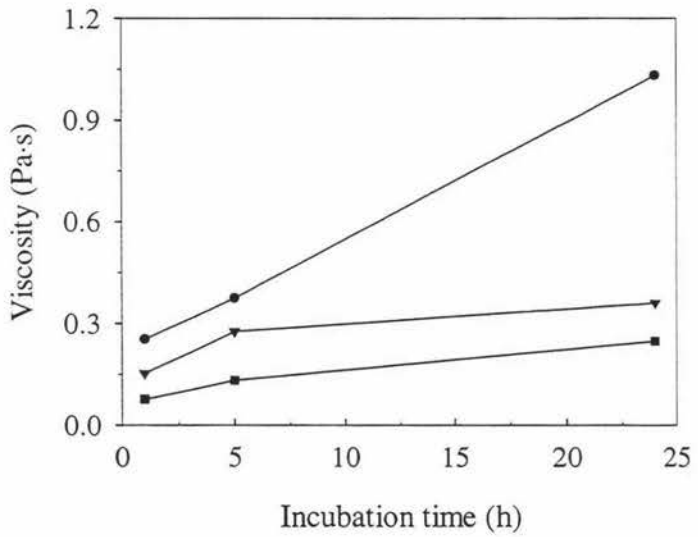


Figure 5.7. Viscosity changes in 10% heat-denatured WPC solution during 1-24 hrs of incubation at 5°C (●), 20°C (▼) and 40°C (■) measured at shear rate of 15.15 s^{-1} (11.74 rpm).

5.6. Discussion

In this chapter, the heating conditions (1% protein solution from UF 1° retentate at pH 7.0 and heated at 80°C for 20 min), determined in Chapter 4, were used in a Massey pilot plant to produce heat-denatured WPC powder. The functional properties of this product were tested. Upon reheating (≥ 70°C), the 12% denatured WPC solutions formed gels although was not as strong as those of the unheated control under the same heating conditions. The denatured WPC solutions formed strong gels upon addition of 15-360 mM $CaCl_2$ followed by incubation for 4 hrs at 40°C, while the unheated WPC solutions did not form gel at any incubation conditions. Upon incubation at 5-40°C without salt addition, the denatured WPC solutions were highly viscous, whereas the unheated WPC solutions did not show any change in viscosity.

A number of studies have been carried out on cold-set gelation of WPI and the effect of environmental conditions on cold-set gelation of denatured whey proteins (Barbut & Foegeding, 1993; Kawamura *et al.*, 1993; McClements & Keogh, 1995; Nakamura *et al.*, 1995; Hongsprabhas & Barbut, 1997a, b; Ju & Kilara, 1998a, b).

Heat-denatured whey proteins are capable of forming gels upon addition of salts or acidulants at ambient temperature. Previous studies were all limited to laboratory scale using relatively expensive WPI powder as the material to produce heated WPI solutions. The present study was carried out at the pilot plant scale, using an online process to produce denatured WPC powder from whey.

The viscosity of heat-denatured WPC produced in the pilot plant was about 21 to 57-fold higher than that of unheated WPC solution depending on protein concentration and incubation conditions. McClements & Keogh (1995) reported a viscosity of 31 mPa·s for a 10% heated WPI solution (90°C, 30 min) at 200 sec^{-1} at 25°C, which was 13.5-fold higher than that of unheated WPI solution. Although it is difficult to compare the absolute viscosity values of whey protein solutions being tested separately, the difference in viscosity at 200 sec^{-1} found in this study was 28-fold higher in 10% denatured WPC solution compared with that of unheated WPC solution at 20°C.

Addition of $CaCl_2$ at concentration ≥ 15 mM induced gelation of 10% heat-denatured WPC solutions at 40°C, but not at 5°C and 20°C. The gel strength and gel opacity increased as $CaCl_2$ concentration was raised from 15 to 360 mM, suggesting larger aggregates were formed by increasing $CaCl_2$ concentration. Gel strength increased rapidly with $CaCl_2$ addition from 15 to 30 mM and then increased at a slower rate from 30 to 240 mM. Further increase in $CaCl_2$ from 240 to 360 mM had no significant effect on gel strength. The results obtained in this study were in agreement with the findings of Barbut (1995).

Heat-denatured WPC was capable of forming gels by reheating without any additives. The gel formed by denatured WPC was more elastic than that formed by unheated control sample under the same heat treatment although the gel was not as strong as that formed by unheated WPC. A different two-step heating process has been described in the previous studies of reheating effect on the gel properties of egg ovalbumin and whey proteins. In that process, addition of salt (Ca^{2+} or Na^{+}) to the pre-heated solution either resulted in cold-gelation (Barbut & Drake, 1996; Hongsprabhas & Barbut, 1998) or resulted in a viscous solution which formed a gel by second heating (Doi, 1993; Murata *et al.*, 1993). In the first case, the already formed cold set WPI gels became harder and more opaque as a result of the second

heating, while in the second case the two-step heating brought clearer gel of ovalbumin or BSA compared to one-step heat-induced gelation. In the present study, reheating, which was carried out in the absence of salt, caused gel formation. The finding that heat-denatured WPC solution could form a gel upon reheating suggested that the WPC powder produced in this study had different gelation properties than preheated WPI as previous research indicated that pre-heated (80°C for 30 min) WPI suspension could not form a gel by reheating without any addition such as Ca^{2+} (Hongsprabhas & Barbut, 1998).

In conclusion, soluble WPC powders containing denatured whey proteins were produced in the pilot plant. The product with cold-gelation ability has potential applications in various foods, such as surimi, pressed ham/bacon, dressing, spreads and bakery products. Heat-denatured WPC solution formed a viscous solution in the absence of additives at 5°C and formed gels upon addition of $CaCl_2$. These are very interesting characteristics for the application as thickening or gelling agents in foods.

CHAPTER 6
PRODUCTION OF DENATURED WPC POWDERS AT ANCHOR PRODUCTS, EDGECUMBE

6.1. Introduction

The heat-denatured WPC powders were produced in the pilot plant at Massey University (Chapter 5). The product was capable of forming viscous solutions or gels upon re-hydration and addition of $CaCl_2$ at incubation temperature (40°C). The successful production of denatured WPC product in a small-scale pilot plant led a possibility of producing denatured WPC powder with superior functionality at an industrial scale.

In addition to Ca-induced gelation, previous studies on cold-set gelation of denatured whey proteins demonstrated that addition of GDL resulted in viscous solutions or gels of WPI solutions at ambient temperatures (Kawamura *et al.*, 1993; Nakamura *et al.*, 1995). The viscosity or gel strength was a function of GDL concentration and incubation conditions.

In this chapter, an attempt was made to produce heat-denatured WPC powder at an industrial scale using the same process conditions applied at the Massey pilot plant. The composition and denaturation level of WPC powders were determined and compared to that of WPC powders produced in the pilot plant at Massey University. The effects of GDL, $CaCl_2$ and NaCl on viscosity and gelation of WPC solutions were investigated at 5-40°C and the possible applications of the heat-denatured WPC were also discussed.

6.2. Pilot plant trials

The processing conditions determined in the preliminary study (Chapter 4) and that used in Massey pilot plant (Chapter 5) were applied to produce heat-denatured WPC powders in the pilot plant at Anchor Products, Edgecumbe. The denatured WPC powders were produced from 1° UF retentate following the procedure described in Section 3.2.5, while the unheated control WPC powder was produced following the same procedure without the heat treatment step prior to UF.

Because the UF pilot plant was not well equipped for the required heat treatment of the trials, it had to use flexible hoses to connect to a nearby milk mineral

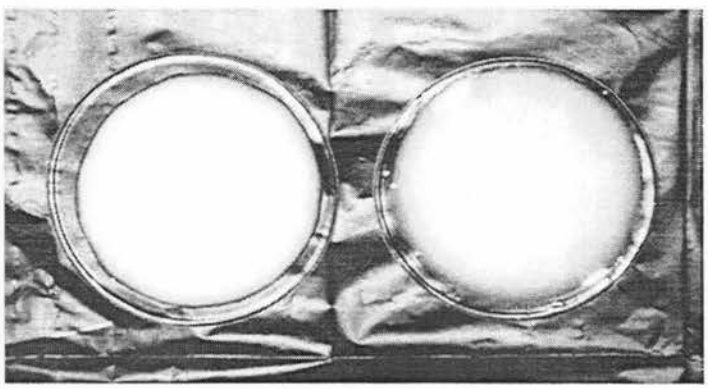
pilot plant. The 1° retentate obtained from the commercial UF plant was manually diluted to 1% protein solution in a reaction tank. Using a NaOH dosing system, the pH of the solution was adjusted to 7.0. The solution was heated to 80°C ± 1°C by steam in a plate heat exchanger and held at 80°C for 20 min. The heated 1% protein solution was then cooled by chilled water in another tubular heat exchanger. However, the cooling facility was not effective enough to cool down the whey protein solution promptly to the required level (< 60°C) to stop the heating process. The whey protein solution ended up being heated more than actually desired.

After cooling to 50°C, the protein solution was pumped into UF line in the pilot plant. During UF/DF, the system temperature was maintained > 50°C. To avoid the denatured whey protein from gel formation during the process, the 2° retentate from UF had to be dried within 4 hrs of production. The 2° retentate was manually delivered to the casein plant to produce WPC powder by the spray dryer. A control run was made using the same processing conditions without the heating step producing undenatured WPC powder.

6.3. Characteristics of WPC powders

6.3.1. Physical characteristics

There were two types of heat-denatured WPC powders produced from each trial. H1015C and H1020C were "coarse" powders collected from the drying chamber of the spray drier, whereas H1015F and H1020F were "fine" powders collected from the bag house (the cyclone by the drier). The separation of powder was based largely on their particle size, and was due to the dryer set-up. Adjustment could have been made to produce 100% fine powder. The WPC powder with fine particles was collected into the bag house by airflow while the powder with relatively large particles stayed in the drying chamber. The "fine" powders look very similar to the unheated WPC powders, U1020C and U1020F, produced without the preheating step. All WPC powders were water-soluble and had no colour difference. The solution prepared from the heat-denatured WPC powder was much more viscous compared with that of unheated WPC powder (Figure 6.1).



a *b*

Figure 6.1. Photograph of 10% heat-denatured (*a*) and unheated (*b*) WPC solutions.

6.3.2. Composition

Same as in the Massey trials, the composition of WPC powders produced from the Edgecumbe trials was consistent within the trials (Table 6.1). Protein contents ranged from 81% to 83%, whereas ash content, total solids and pH value varied 0.1%, 2.1% and 0.08 unit, respectively. In addition, the composition of WPC powders produced from the Edgecumbe trials was comparable to that of WPC powders produced from the Massey trials. The results indicated that WPC powder produced in a small-scale pilot plant could be reproduced in a large-scale pilot plant.

Table 6.1. Composition of WPC powders produced in Edgecumbe pilot plant

Trial No.	WPC powder	Protein (N × 6.38) (%)	Ash (%)	Total solids (%)
5	H1015C	83.17 ± 0.38	2.02 ± 0.05	95.20 ± 0.20
5	H1015F	83.44 ± 0.48	1.96 ± 0.02	93.72 ± 0.23
6	H1020C	81.44 ± 0.17	2.07 ± 0.01	93.10 ± 0.11
6	H1020F	80.95 ± 0.13	2.05 ± 0.01	93.18 ± 0.13
7	U1020C	83.47 ± 0.52	1.96 ± 0.13	94.13 ± 0.22
7	U1020F	82.64 ± 0.38	1.97 ± 0.12	94.29 ± 0.19

H = heated; U = unheated; C = coarse powder; F = fine powder.

6.3.3. Extent of whey protein denaturation

The native-PAGE patterns of 1% protein solutions prepared from WPC powders are shown in Figure 6.2. The pattern was essentially similar to that of WPC solutions from the Massey trials (Figure 5.1). In comparison with the unheated control WPC solution (lane 3), the band intensities of native whey proteins in the heated WPC solution (lane 1 & 2) were diminished. In particular, bands of α-La and BSA almost disappeared. The bands representing intermediate aggregates could not be seen clearly in the heated WPC solutions, in contrast to the heated retentate solution (Figure 4.2). Instead, higher proportions of high molecular weight aggregates were caught on the top of stacking and resolving gels in the heated WPC solution (lane 1& 2) than that in the unheated control sample (lane 3). The presence of larger aggregates and absence of small intermediate aggregates suggested that the heat treatment had gone beyond the required level or drying influenced the nature of aggregation.

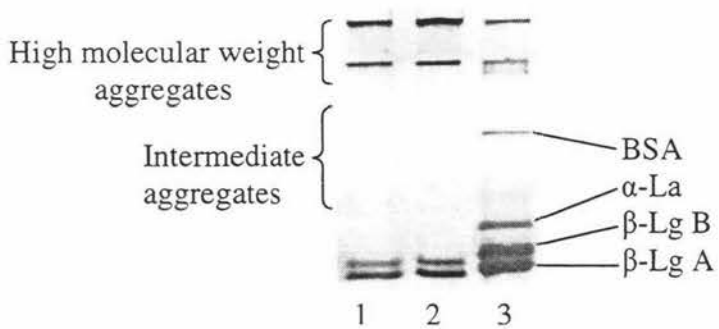


Figure 6.2. Native-PAGE patterns of 1% whey protein solution prepared from WPC powders produced from trial 5 (lane 1), trial 6 (lane 2) and trial 7 (lane 3) at Anchor Products, Edgecumbe.

The data obtained from the densitometric scanning of the gels confirmed the visual observation of the native-PAGE. As expected, similar denaturation levels of whey proteins were achieved between Edgecumbe trials (Table 6.2). The denaturation of β-Lg, α-La and BSA in the coarse powders did not differ from that in the fine powders. Loss of β-Lg A and β-Lg B was comparable to that found in the WPC powders from the Massey trials. However, α-La was denatured more extensively than

β-Lg in the WPC solutions from Edgecumbe trials, which was contrary to the results observed in the heated 1% protein solution from UF retentate or heated WPC solutions from Massey trials. In the heated retentate solution, the rate of loss of β-Lg was greater than that of α-La, while similar denaturation level of β-Lg and α-La was found in the WPC solutions from the Massey trials. The reason for the different heat sensitivity of β-Lg and α-La in heated retentate and WPC solutions from different pilot plants is not obvious. Different UF and drying system used in the process might have affected the whey protein denaturation. Moreover, the different heating extent, i.e. the cooling after heating at Edgecumbe pilot plant was not prompt, could have been significant.

Table 6.2. Denaturation of whey proteins in WPC powders (%)

Whey protein	H1015C	H1015F	H1020C	H1020F
β-Lg A	73.8 ± 0.8	74.8 ± 0.7	70.2 ± 0.6	69.1 ± 0.4
β-Lg B	75.9 ± 0.9	77.1 ± 0.5	72.3 ± 0.7	72.4 ± 0.8
α-La	93.3 ± 0.4	95.8 ± 0.6	93.2 ± 0.6	86.2 ± 0.2
BSA	100	100	100	100

The denaturation levels of whey proteins in the heat-denatured WPCs were also quite similar between the coarse and fine powders from the both trials. The following functional properties of WPC were determined using the coarse powders produced from drying chamber in spray drier.

6.4. Functional properties of heat-denatured and unheated WPC

6.4.1. Viscosity of WPC solutions without additives

Heat-denatured or unheated WPC solutions (5%, 10% and 15%, protein basis) were prepared (Section 3.2.7) and incubated at 5°C, 20°C or 40°C for 1, 5 or 24 hrs. The viscosities of these solutions were measured over a wide range of shear rate (Section 3.2.9) at the corresponding temperatures. To keep consistent with the preparation of samples from the Massey trials, an attempt was made to prepare 15%

protein solution of WPC. However, the heat-denatured WPC powder could not be dissolved thoroughly due to the high viscosity of the solution.

The viscosity patterns of heat-denatured WPC solutions as related to shear rate at 5°C (Figure 6.3) were quite similar to those at 20°C or 40°C (Appendix 6.1 & 6.2). Generally the heat-denatured WPC solutions exhibited a thixotropic character (shear thinning). The viscosity increased with increasing protein concentration, incubation time and decreased with incubation temperature. It was interesting that 12% protein solution of the heat-denatured WPC formed a weak gel when incubated at 40°C for 24 hrs without any additives.

The unheated WPC solutions had much lower viscosity, 25 to 73-fold lower, than the denatured WPC solutions at the same shear rate, protein concentration, incubation temperature and time. The viscosity vs shear rate plots for the denatured WPC solutions from the Edgecumbe trials (Figure 6.3) were comparable to those of the denatured WPC solutions from the Massey trials (Figure 5.6).

The rate of increase in viscosity with protein concentration and incubation time, or a decrease in viscosity with temperature observed in the denatured WPC solutions prepared from H1015 was not different from that of H1020, which was also comparable to that found in the denatured WPC solutions from the Massey trials.

6.4.2. Effect of GDL addition on WPC solutions

In heat-denatured or unheated WPC solutions (Section 3.2.7), glucono-δ-lactone (GDL) powder (0.5%, 1% & 2%, w/w) was added at 5°C, 20°C or 40°C to give 10% protein solutions of WPC. The solutions were incubated at corresponding temperatures for ~ 4 hrs. Under different incubation conditions, the heat-denatured WPC formed viscous solution or gel, whereas the unheated WPC solutions did not change viscosity or form gel. The viscosity or gel strength of the incubated WPC solutions were tested and the pH of each solution was measured before the measurement of viscosity or gel strength.

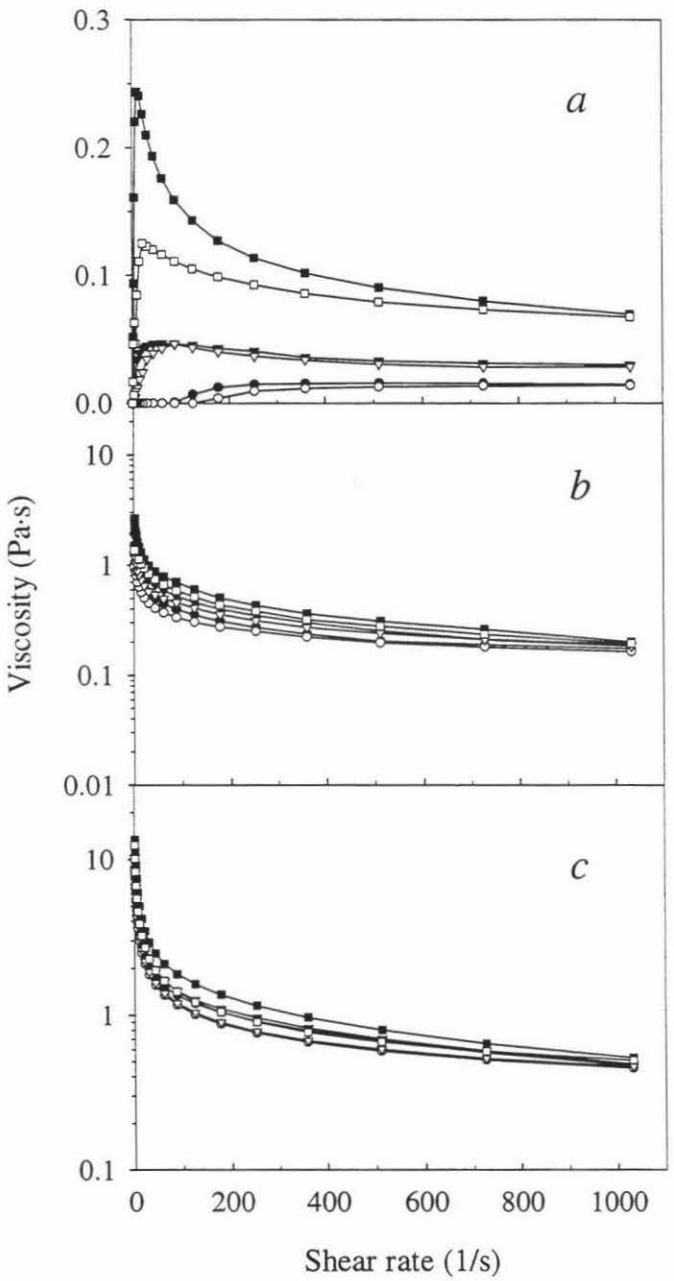


Figure 6.3. Viscosity of 5% (*a*), 10% (*b*) and 12% (*c*) heat-denatured WPC solutions (H1015) incubated at 5°C for 1 h (● ○), 5 h (▼ ▽) or 24 h (■ □) as a function of shear rate. Shear rate increased from 0 to 1032.5 sec^{-1} (800 rpm) (● ▼ ■) and decreased from 1032.5 to 0 sec^{-1} (○ ▽ □) in a step manner.

When GDL powder was added to the WPC solutions, GDL hydrolysed to gluconic acid, which resulted in a reduction of solution pH. Higher GDL concentration resulted in lower pH values of the solutions (Figure 6.4). Decrease in pH with incubation time was similar in both denatured and unheated WPC solutions. The pH decrease was dramatic in the first hour and not much change was observed thereafter. The incubation temperature also affected the rate of acidification. Decrease in pH was faster at high incubation temperatures (Appendix 6.3). The lowest pH value of 4.0 was obtained when the heat-denatured WPC solution with 2% GDL was incubated at 40°C for 4 hrs.

The heat-denatured WPC solution firstly appeared as a highly viscous solution when the pH decreased to ~ 5.0 during the incubation and then the solution formed gels when the pH lowered to ≤ 4.9. However, the unheated WPC remained as solution even at the final pH of ~ 4.0. The different results between the denatured and unheated WPC upon addition of GDL indicated that preheating of WPC solution, resulting in aggregate formation of whey proteins, is essential for GDL-induced viscous solutions or gels.

The solution-gel transition of the 10% denatured WPC depended on the GDL concentration, incubation temperature and time (Table 6.3). Higher GDL concentration and higher temperature resulted in faster gelation presumably due to the increase rate of hydrolysis of GDL, i.e. higher level of gluconic acid and lower pH level. The WPC solutions formed gels upon addition of 1-2% GDL after over 1 h incubation at 20-40°C, whereas upon addition of 0.5% GDL, gels were only formed after incubation at 40°C for 4 hrs.

At a given GDL concentration, the gelation time increased with decreasing incubation temperature. For example, gels were formed upon addition of 2% GDL after 1 h and 2 h incubation at 40°C and 30°C, respectively. At 20°C, gelation only occurred upon addition of 2% GDL at 40°C for 4 hrs, and the gel was too weak to be tested. No gelation occurred at 5°C during ~ 4 h incubation.

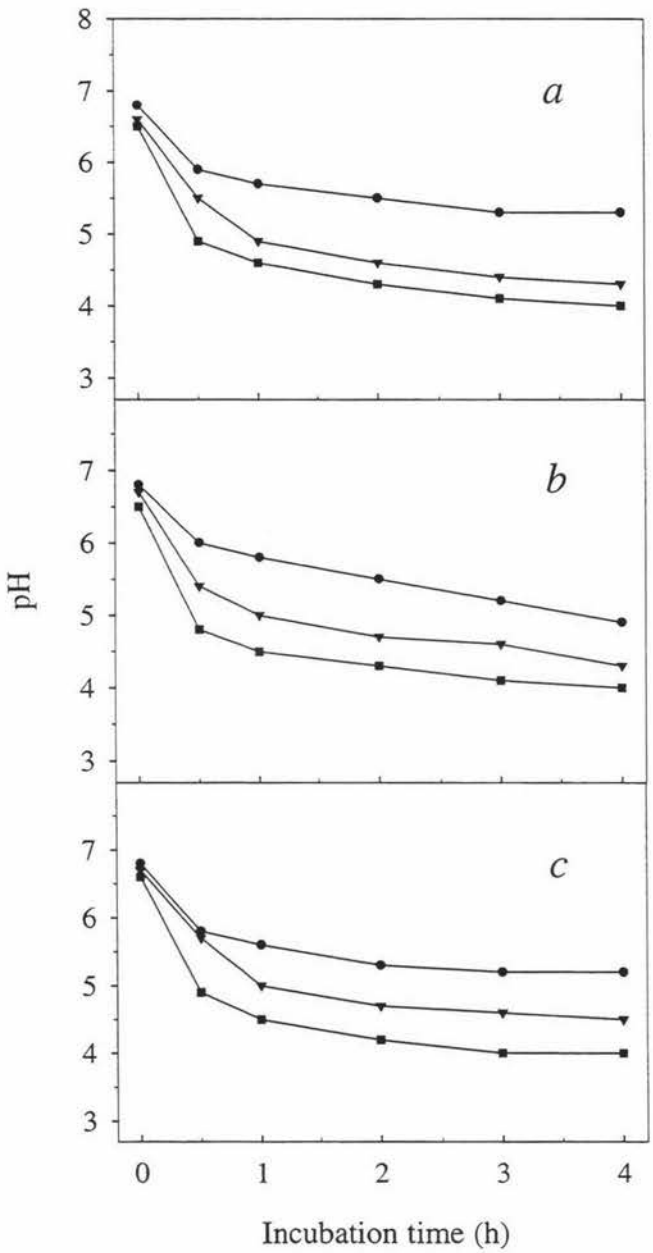


Figure 6.4. Comparison of pH changes in 10% heat-denatured WPC solutions prepared from H1015 (*a*), H1020 (*b*) and in 10% unheated WPC solution prepared from U1020 (*c*) upon addition of 0.5% (●), 1%(▼) and 2% (■) GDL at 40°C.

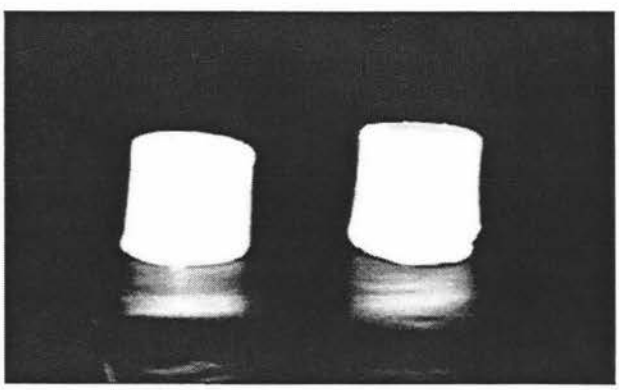
Table 6.3. Formation of viscous solutions or gels from WPC solutions (H1020) upon addition of GDL under different incubation conditions

	Denatured WPC					Unheated WPC
40°C	0.5 h	1 h	2 h	3 h	4 h	~ 4 h
0.5% GDL	V-sol	V-sol	V-sol	V-sol	gel	Sol
1% GDL	V-sol	V-sol	gel	gel	gel	Sol
2% GDL	V-sol	gel	gel	gel	gel	Sol
30°C	0.5 h	1 h	2 h	3 h	4 h	~ 4 h
0.5% GDL	V-sol	V-sol	V-sol	V-sol	V-sol	Sol
1% GDL	V-sol	V-sol	V-sol	V-sol	gel	Sol
2% GDL	V-sol	V-sol	V-sol	V-sol	gel	Sol
20°C	0.5 h	1 h	2 h	3 h	4 h	~ 4 h
0.5% GDL	V-sol	V-sol	V-sol	V-sol	V-sol	Sol
1% GDL	V-sol	V-sol	V-sol	V-sol	V-sol	Sol
2% GDL	V-sol	V-sol	V-sol	V-sol	gel	Sol
5°C	0.5 h	1 h	2 h	3 h	4 h	~ 4 h
0.5% GDL	V-sol	V-sol	V-sol	V-sol	V-sol	Sol
1% GDL	V-sol	V-sol	V-sol	V-sol	V-sol	Sol
2% GDL	V-sol	V-sol	V-sol	V-sol	V-sol	Sol

- V-sol: viscous solution

Visually, the GDL-induced gels of heat-denatured WPC solutions were milky, translucent or opaque, smooth and custard-like. There was little difference between the gels formed from H1015 and H1020 (Figure 6.5). The gels made with higher GDL concentration at high temperature or longer incubation time showed a slight spontaneous syneresis. Spontaneous syneresis is defined as shrinkage of a gel without

the application of any external forces (e.g. centrifugation), and this occurs concomitantly with expulsion of liquid or whey separation. This phenomenon is a common defect in acid-induced gel and is related to instability of the gel network, i.e. large scale rearrangement (Lucey & Singh, 1998).



a *b*

Figure 6.5. Photograph of gel cylinders from 10% heat-denatured WPC solutions prepared from H1015 (*a*) and H1020 (*b*) upon addition of 1% GDL at 40°C for 4 hrs.

Effect of GDL on viscosity of denatured WPC solutions

Viscosity of heat-denatured WPC solutions showed a shear rate, GDL concentration, temperature and time dependence. The viscosity of the two denatured WPC solutions, prepared from H1015 and H1020, decreased with shear rate at 40°C (Appendix 6.4), consistent with the viscosity pattern of heat-denatured WPC solutions in the absence of additive (Appendix 6.2). By comparison, the WPC solutions from H1020 had slightly higher viscosity values than those from H1015. The unheated WPC solution (U1020), upon addition of GDL, had much lower viscosity than the heat-denatured WPC solutions. The maximum viscosity value of the unheated WPC solution determined was only 20.8 mPa·s at 40°C. The values were almost equal to those of original unheated WPC solution, indicating that addition of GDL had no effect on viscosity of the unheated WPC solution.

To compare the effects of GDL concentration and time on viscosity of denatured WPC solutions from H1015 and H1020, the viscosity of 10% denatured WPC solutions measured at shear rate of 1.29 sec^{-1} were shown in Figure 6.6. The viscosity of denatured WPC solutions increased with increasing GDL concentration and incubation time at room temperature (20°C). Similar trends were found for the two WPC solutions prepared from H1015 and H1020. Rapid increase in viscosity with incubation time occurred at higher GDL concentrations (1% & 2%), whereas the viscosity of WPC solutions varied very little upon addition of 0.5% GDL.

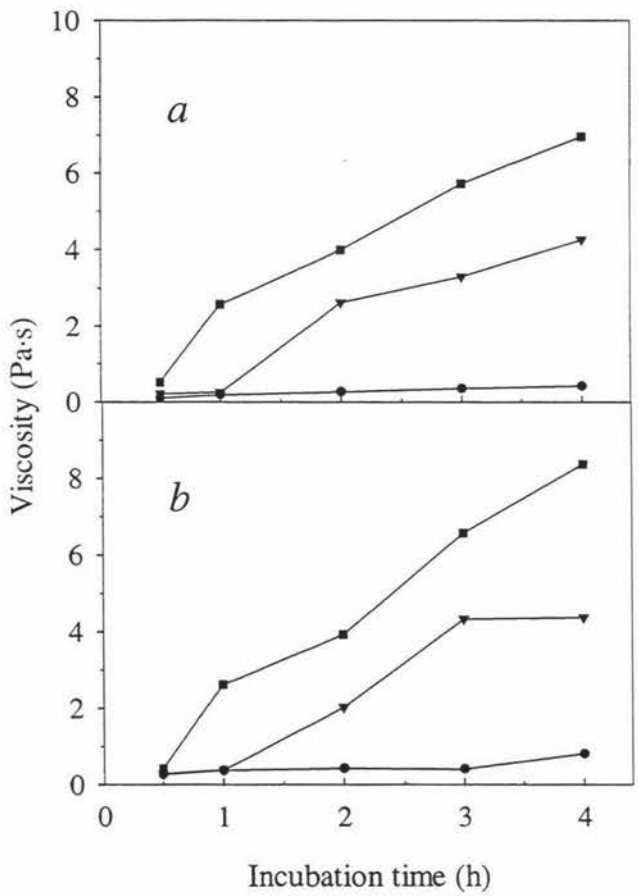


Figure 6.6. Effect of GDL concentration and incubation time on viscosity of 10% heat-denatured WPC solutions prepared from H1015 (*a*) and H1020 (*b*) measured at shear rate of 1.29 sec^{-1}. The solutions were incubated with 0.5% (●), 1% (▼) and 2% (■) GDL at 20°C.

In addition to GDL concentration, incubation temperature had a significant effect on viscosity of the denatured WPC solutions. The viscosity of denatured WPC solutions upon addition of 0.5% GDL under different incubation temperatures is shown in Figure 6.7. Viscosity increased with incubation temperature and time. The increase in viscosity was much more rapidly at 40°C and 30°C compared to 20°C and 5°C. At 40°C, the denatured WPC solution formed a gel after incubation for 4 hrs upon addition of 0.5% GDL. The results revealed that increasing temperature could increase viscosity and accelerate gelation of the denatured WPC solutions with GDL.

As mentioned before, the pH change of WPC solutions due to addition of GDL occurred largely in the first hour of incubation (Figure 6.4). However, the change in viscosity of denatured WPC solutions was obvious after incubation for 3 hrs (Figure 6.7). The fact that viscosity change occurred much later than pH change indicated that a certain time was required to allow the interactions to take place between the aggregates of whey proteins, which gave rise to viscosity changes or gel formation.

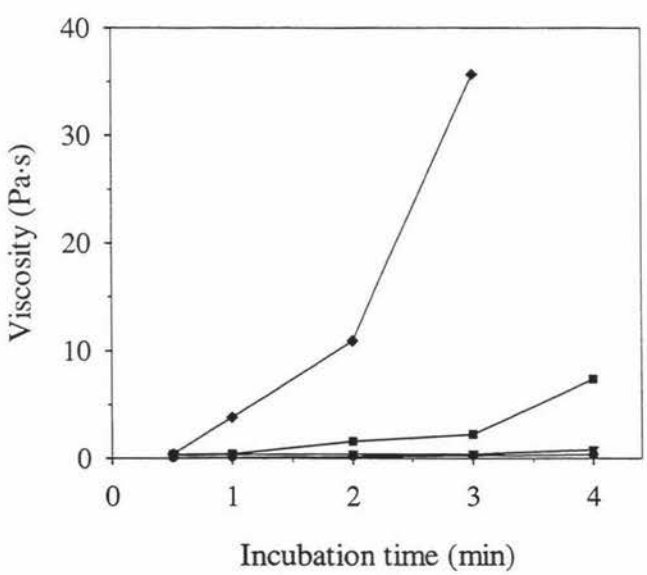


Figure 6.7. Effect of incubation temperature and time on viscosity of 10% heat-denatured WPC solution (H1020) upon addition of 0.5% GDL at 5°C (●), 20°C (▼), 30°C (■) and 40°C (◆) as measured at shear rate of 1.29 sec^{-1}.

Effect of GDL on gelation of denatured WPC solutions

Both the denatured WPC solutions (10% protein, w/v) formed gels upon addition of 1-2% GDL at 20-40°C over 1 h incubation, whereas gels were only formed upon addition of 0.5% GDL after incubation at 40°C for 4 hrs (Table 6.3). The pH value at initial gel formation (≤ 4.9) was lower than those in previous reports, where 6% and 8% heat-denatured WPI solutions started to form gels at pH 5.8 and 5.3 upon addition of 1-2% and 0.4-2% GDL, respectively (Kawamura *et al.*, 1993; Ju & Kilara, 1998a).

GDL concentration, incubation temperature and time affected the rate of GDL-induced gelation as well as the gel strength. Higher GDL concentration and higher incubation temperature resulted in fast gelation, presumably due to the high rate of GDL hydrolysis. The gel strength increased with increasing GDL concentration from 0.5% to 1% and then decreased with increasing GDL concentration from 1% to 2% (Figure 6.8). At a given GDL concentration, gels formed at higher temperature were stronger than that formed at lower temperature (Appendix 6.5). In comparison, the denatured WPC solution from H1020 formed stronger gels than did H1015. However, the change in gel strength as a function of temperature and GDL concentration was generally similar in H1015 and H1020, i.e. a decrease with increasing GDL concentration or decreasing temperature. A similar effect of temperature and GDL concentration on fracture stress was observed by Kawamura *et al.* (1993) for gels formed by adding 1-2% GDL to heated WPI solutions at 20-50°C.

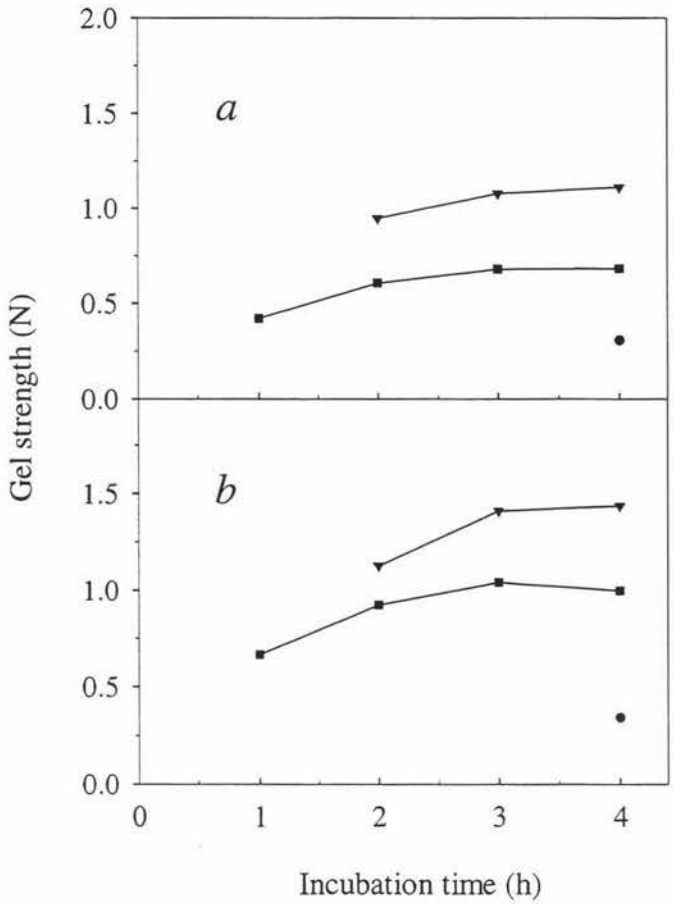


Figure 6.8. Effect of GDL concentration and incubation time on gel strength of 10% heat-denatured WPC solutions prepared from H1015 (*a*) and H1020 (*b*) at 40°C. GDL concentration was 0.5% (●), 1%(▼) and 2% (■).

6.4.3. Effect of $CaCl_2$ addition on WPC solutions

Appropriate amountS of 1.2 M $CaCl_2$ solution were added to heat-denatured or unheated WPC solutions (Section 3.2.7) to give final concentrations of 10-120 mM $CaCl_2$ in 10% protein solutions of WPC (Section 3.2.8). The solutions were adjusted to pH 7.0 and incubated at different temperatures for ~ 4 hrs. Under different $CaCl_2$ concentrations and incubation temperatures, the 10% heat-denatured WPC solutions formed viscous solutions or gels, whereas the unheated WPC solutions did not show any change in viscosity or form gels (Table 6.4).

Table 6.4. Formation of viscous solutions or gels from WPC solutions (H1020) upon addition of $CaCl_2$ under different incubation conditions

	Denatured WPC					Unheated WPC
40°C	0.5 h	1 h	2 h	3 h	4 h	~ 4 h
10 mM $CaCl_2$	V-sol *	V-sol	V-sol	V-sol	gel	Sol
20 mM $CaCl_2$	V-sol	V-sol	gel	gel	gel	Sol
30 mM $CaCl_2$	V-sol	gel	gel	gel	gel	Sol
60 mM $CaCl_2$	gel	gel	gel	gel	gel	Sol
90 mM $CaCl_2$	gel	gel	gel	gel	gel	Sol
120 mM $CaCl_2$	gel	gel	gel	gel	gel	Sol
20°C	0.5 h	1 h	2 h	3 h	4 h	~ 4 h
10 mM $CaCl_2$	V-sol	V-sol	V-sol	V-sol	V-sol	Sol
20 mM $CaCl_2$	V-sol	V-sol	V-sol	V-sol	gel	Sol
30 mM $CaCl_2$	V-sol	V-sol	gel	gel	gel	Sol
60 mM $CaCl_2$	gel	gel	gel	gel	gel	Sol
90 mM $CaCl_2$	gel	gel	gel	gel	gel	Sol
120 mM $CaCl_2$	gel	gel	gel	gel	gel	Sol
5°C	0.5 h	1 h	2 h	3 h	4 h	~ 4 h
10 mM $CaCl_2$	V-sol	V-sol	V-sol	V-sol	V-sol	Sol
20 mM $CaCl_2$	V-sol	V-sol	V-sol	V-sol	V-sol	Sol
30 mM $CaCl_2$	V-sol	V-sol	V-sol	V-sol	gel	Sol
60 mM $CaCl_2$	V-sol	V-sol	V-sol	V-sol	gel	Sol
90 mM $CaCl_2$	V-sol	V-sol	gel	gel	gel	Sol
120 mM $CaCl_2$	gel	gel	gel	gel	gel	Sol

* V-sol: viscous solution

The viscous solutions or gels formed upon addition of $CaCl_2$ to 10% denatured WPC solutions prepared from H1015 and H1020 were translucent or opaque, depending on incubation conditions. The solutions generally became viscous at lower $CaCl_2$ concentrations and formed gels at higher $CaCl_2$ concentrations. Viscosity of 10% denatured WPC solutions in the presence of $CaCl_2$ increased with incubation time (Figure 6.9).

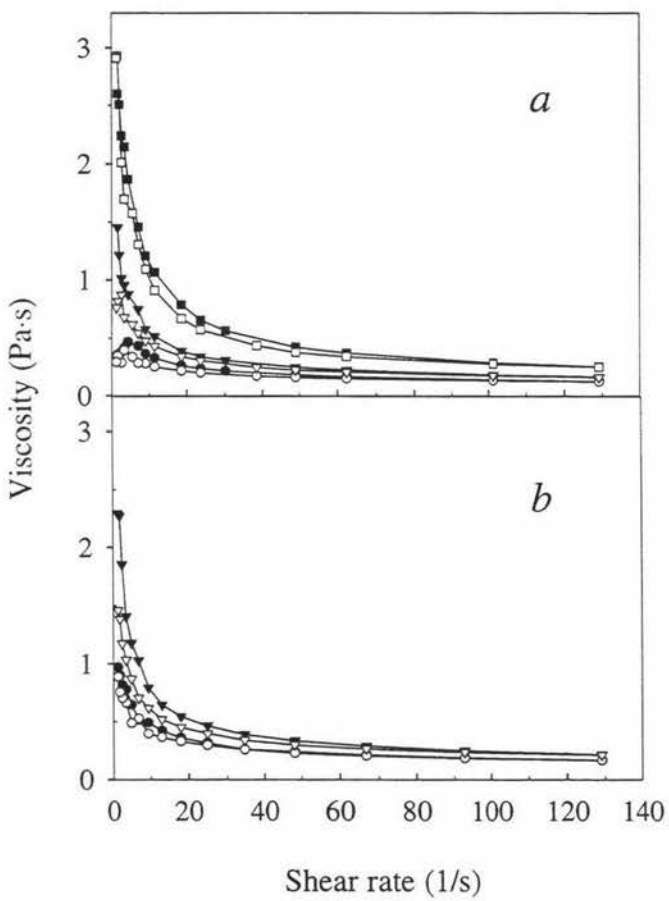


Figure 6.9. Viscosity of 10% heat-denatured WPC solutions prepared from H1015 (*a*) and H1020 (*b*) incubated with 10 mM $CaCl_2$ at 20°C for 0.5 h (● ○), 4 h (▼ ▽) and 24 h (■ □) as a function of shear rate. Shear rate increased from 1.29 to 129.1 sec^{-1} (● ▼ ■) and then decreased from 129.1 to 1.29 sec^{-1} (○ ▽ □) in a stepwise manner.

The increase in viscosity with incubation time was comparable between H1015 and H1020, although the viscosity of H1015 was lower than that of H1020 at the corresponding incubation conditions. To some extent, the gels were rougher than that of GDL-induced gel. At higher $CaCl_2$ concentration (≥ 30 mM), the gel surface had visible cracks and some liquid separation (syneresis).

Effect of $CaCl_2$ on viscosity of denatured WPC solutions

$CaCl_2$ level had a significant effect on the viscosity of 10% heat-denatured WPC solutions from both trials. Viscosity increased with incubation time at all $CaCl_2$ concentrations until the solution formed a gel. At a given temperature (e.g. 20°C), the viscosity at higher $CaCl_2$ concentrations was higher than that at lower $CaCl_2$ concentrations (Appendix 6.6). The rate of increase in viscosity with incubation time was greater at high $CaCl_2$ concentrations than that at low concentrations.

The viscosity of denatured WPC solution upon addition of $CaCl_2$ also had temperature dependence (Figure 6.10). At short incubation time, the initial value of viscosity was higher at lower temperatures than that at higher temperatures. However, the rates of increase in viscosity with incubation time were higher at higher temperatures than that at lower temperatures. At 40°C, the viscosity increased much more rapidly compared to that at 5°C and 20°C. This resulted in higher viscosity at 40°C than that at 20°C and 5°C after 4 h incubation. The increasing viscosity with temperature was contrary to the previous observation (Section 6.4.1), where in the absence of additives, the viscosity of denatured WPC solutions decreased as temperature increased. In the presence of $CaCl_2$, the viscosity is the result of balance of hydrophobic interactions and electrostatic repulsion between the protein aggregates. It has been suggested that when $CaCl_2$ is added to the WPC solution containing linear filament-type protein aggregates, the electrostatic repulsion is shielded, which causes the aggregates to come together to form strands (Bryant & McClements, 1998).

The increase in viscosity with incubation temperature and time was very much dependent on shear rate. The rate of increase in viscosity was greater at lower shear rate than that at higher shear rate. This is because that high shear rate applied breaks the protein aggregates existing in the heat-denatured WPC solutions.

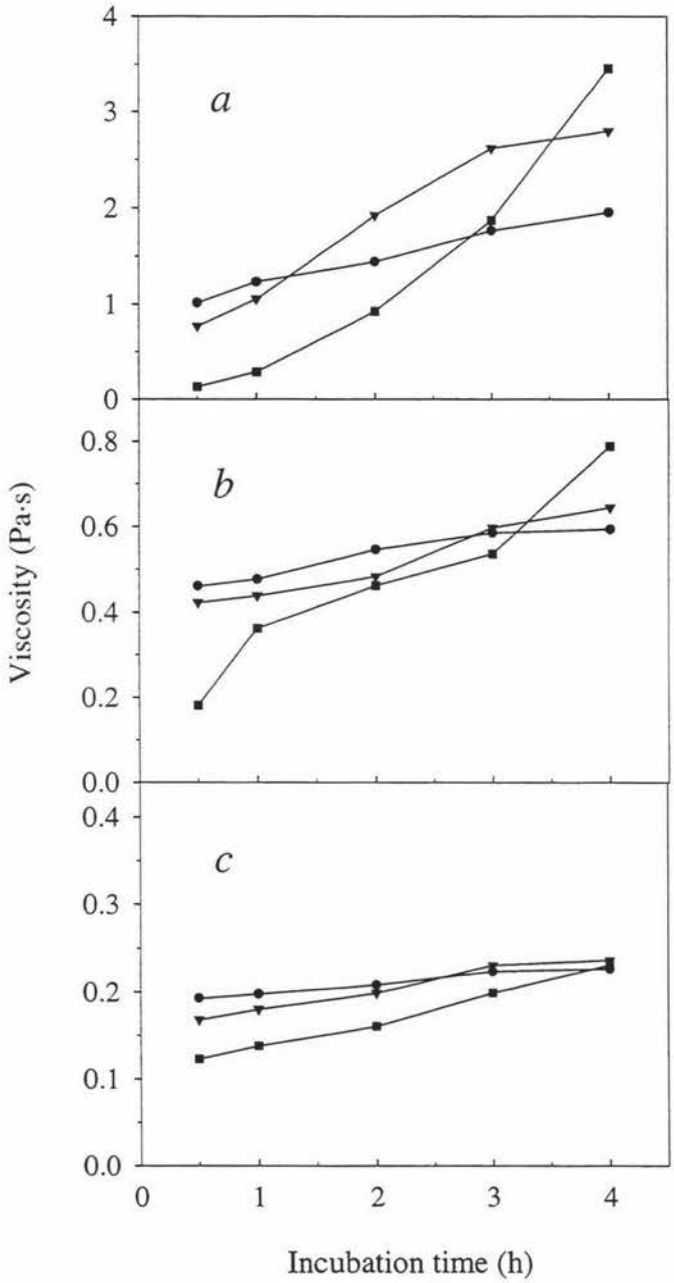


Figure 6.10. Effect of incubation temperature and time on viscosity of 10% heat-denatured WPC incubated with 10 mM $CaCl_2$ at 5°C (●), 20°C (▼) and 40°C (■) as measured at shear rate of 1.29 (*a*), 12.9 (*b*) and 129 (*c*) sec^{-1}.

Effect of $CaCl_2$ on gelation of denatured WPC solutions

Increasing $CaCl_2$ from 10 to 120 mM resulted in an increase in gelation rate of 10% heat-denatured WPC solutions at all incubation temperatures (Table 6.4). Lower $CaCl_2$ concentration (10 mM) did not induce gelation of WPC solutions from the both trials (H1015 & H1020). Gels were formed at $CaCl_2 \geq 20$ mM; the gel strength varied with incubation temperature and time. Gel strength of 10% denatured WPC solutions increased with $CaCl_2$ concentration over incubation time (Figure 6.11). However, the strength of gels formed with 30 mM $CaCl_2$ was comparable to that formed with 60 mM $CaCl_2$. The extent of increase in gel strength with incubation time was greater at higher concentrations than that at lower concentrations, probably due to the effect of $CaCl_2$ on protein-protein interactions.

Gel appearance also changed with $CaCl_2$ concentration. On increasing $CaCl_2$ concentration, gels became progressively more opaque and less elastic, which was consistent with the observation of Barbut (1995). In addition, it was observed that some water was separated from gel with incubation at higher $CaCl_2$ level ($\geq$ 60 mM), suggesting the WHC decreased with increasing in $CaCl_2$ concentration.

In comparison, WPC solutions from H1020 formed gels faster and harder than did that from H1015 at the same $CaCl_2$ concentration, indicating the former had stronger gelling ability. For example, H1020 formed gels at 20 mM $CaCl_2$ but H1015 did not. H1020 formed gels at 30 mM $CaCl_2$ after 2 h incubation at 20°C, while H1015 formed gels at the same concentration of $CaCl_2$ after 3 h incubation.

Gel strength increased with increasing temperature and the extent of increase in gel strength with $CaCl_2$ concentration was greater at higher temperatures (Appendix 6.7). This is probably because hydrophobic interactions become the most important attractive force when Ca^{2+} act as counter-ions, which shield the electrostatic repulsion between the electrically charged filaments and cause them to aggregate. The magnitude of hydrophobic interactions increased with temperature (Baldwin, 1986), and so increase in viscosity or gelation was expected to occur more rapidly at higher temperatures. In addition, the molecules are moving rapidly at higher temperatures and will therefore collide with one another more frequently, which also contribute to an increase in gelation rate (McClements & Keogh, 1998).

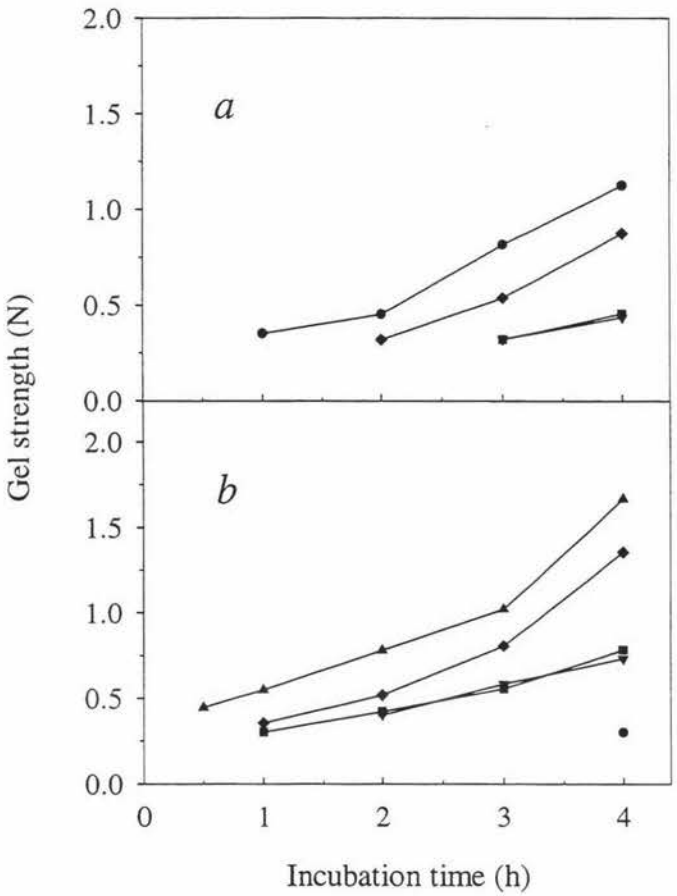


Figure 6.11. Effect of $CaCl_2$ concentration and incubation time on gel strength of 10% heat-denatured WPC solutions of H1015 (*a*) and H1020 (*b*) at 20°C. $CaCl_2$ concentration was added at 20 (●), 30 (▼), 60 (■), 90 (◆) and 120 (▲) mM.

6.4.4. Effect of NaCl addition on WPC solutions

Appropriate amounts of 4 M NaCl solution were added at 20°C to give final concentrations of 20-400 mM NaCl in 10% protein solutions of WPC (Section 3.2.8). The solutions were adjusted to pH 7.0 and incubated at 20°C for ~ 4 hrs or 24 hrs. With different NaCl concentrations, the denatured WPC solutions formed viscous solutions after 4 h incubation, whereas the unheated WPC solutions showed no change in viscosity. When prolonging the incubation time to 24 hrs, gelation of the denatured WPC solutions occurred at NaCl ≥ 100 mM. The gels were translucent or

opaque, rougher than GDL-induced gel but smoother than Ca-induced gel. The unheated controls were incubated with NaCl under the same conditions and no gelation was observed.

Viscosity of 10% denatured WPC solutions incubated with NaCl at 20°C was also showing a typical thixotropic characteristic (Appendix 6.8). At a given shear rate, the viscosity increased with incubation time and NaCl concentration (Figure 6.12). The increase with incubation time was faster at higher NaCl concentrations than that at lower NaCl concentrations.

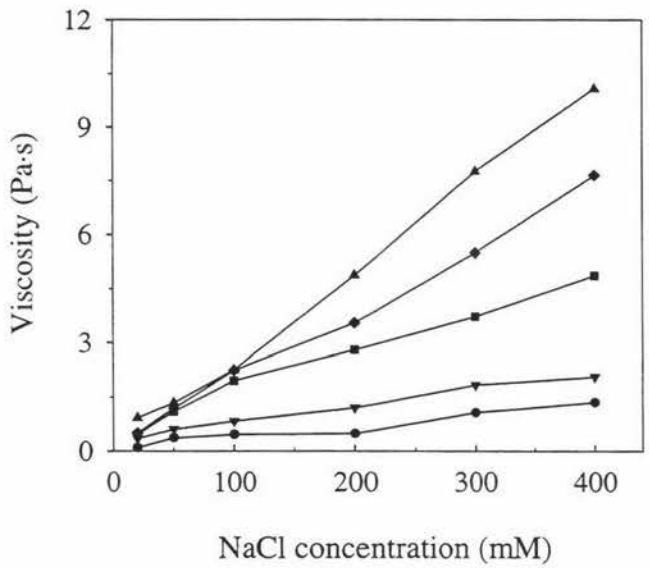


Figure 6.12. Effect of NaCl concentration on viscosity of 10% heat-denatured WPC solutions incubated at 20°C for 0.5 h (●), 1 h (▼), 2 h (■), 3 h (◆) and 4 h (▲) as measured at shear rate of 1.29 sec^{-1}.

Increasing NaCl concentration during gelation of 10% denatured WPC solutions resulted in increasing gel strength (Figure 6.13). At 100 mM NaCl, a very weak gel was formed indicating that this concentration is very close to the minimum Na required to induce cold set gelation of 10% heat-denatured WPC. The minimum NaCl level of 100 mM required to form a gel, found in the present study, was higher

than that reported by Barbut & Drake (1997) and Ju & Kirala (1998a), who used 75 mM and 50 mM NaCl to induce gelation of 10% and 8% heat-denatured WPI solutions. The difference was due to different whey protein systems and different degree of whey protein denaturation.

The relative increase in gel strength was higher at low NaCl concentrations compared to that at high NaCl concentrations. Increasing NaCl concentration from 100 to 200 mM quickly increased gel strength, which was in agreement with the results of Barbut & Drake (1997) and Ju & Kirala (1998a). Further addition of NaCl up to 400 mM only slightly further increased the gel strength.

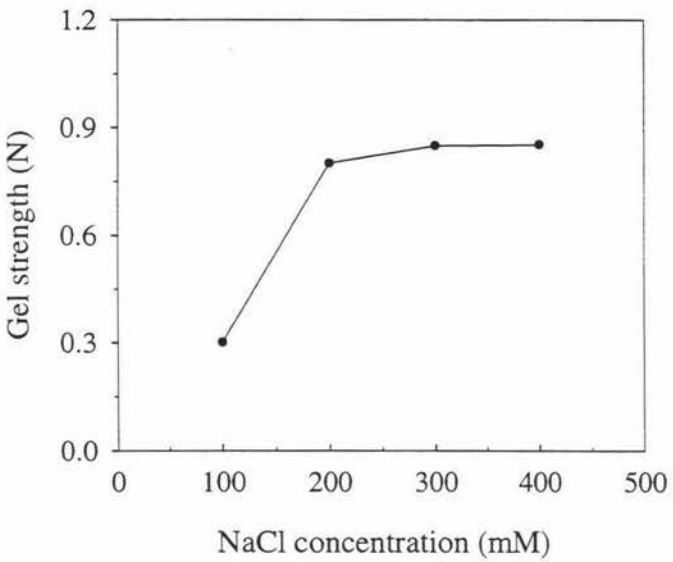


Figure 6.13. Effect of NaCl concentration on gel strength of 10% heat-denatured WPC solutions incubated at 20°C for 24 hrs.

6.5. Discussion

Heat-denatured (H1015 & H1020) and unheated (U1020) WPC powders were produced in the UF pilot plant at Anchor Products, Edgecumbe using the same process conditions applied in the pilot plant at Massey University. The composition and the extent of protein denaturation were determined. The functional properties of

these products were also determined in the absence or presence of GDL, $CaCl_2$ or NaCl after incubation at 5-40°C for various times.

The WPC powders produced at Edgecumbe were similar in composition and extents of protein denaturation to that of WPC powders produced at Massey pilot plant. In the absence of additive, the viscosity of denatured WPC solutions increased with protein concentration, incubation temperature and time. Upon addition of GDL, $CaCl_2$ or NaCl, the denatured WPC solutions formed viscous solutions or gels depending on incubation conditions. By contrast, the unheated WPC solutions did not show any change in viscosity or form gels under the same incubation conditions.

In denatured WPC, proteins have exposed their reactive sites, i.e. hydrophobic and thiol groups and formed intermolecular bonds between the denatured whey proteins, which lead to the formation of aggregates. These aggregates form viscous solutions. At pH 7.0, the denatured whey proteins are negatively charged, and the association of aggregates formed is hindered by electrostatic repulsive forces. This results in less number of collisions and less number of bond formation between the protein aggregates. When GDL was added to the heat-denatured WPC solutions, the net charge on the surface of the aggregates was reduced by acidification. This would allow protein aggregates to come together closer to form bonds by hydrophobic interactions and sulfhydryl/disulfide exchange. In the case of addition of salts (Ca or Na), the electrostatic repulsive forces between the soluble protein aggregates are weakened by shielding effects of counter ions. The Ca^{2+} or Na^{+} mask the negatively charged protein molecules resulting in more number of collisions and more number of bond formation, which lead to gel formation.

These changes apparently depend on the GDL or salt concentration, incubation temperature and time, and the nature of the cation. On the other hand, the fact that no viscous solution or gelation occurred in the unheated WPC solutions by addition of salts or GDL clearly indicated that pre-heating resulting in protein denaturation and "soluble" aggregate formation is an essential step for cold-set gelation.

Results from the present study clearly indicated that the effect of $CaCl_2$ on properties of heat-denatured WPC solutions or gels differed from that of NaCl. Salts alter the interactions between protein filaments in a number of different ways. Both monovalent (Na^{+}) and divalent (Ca^{2+}) salts act as counter-ions, which shield the

electrostatic repulsion between the electrically charged filaments and cause them to aggregate (Kinsella *et al.*, 1989; Wang & Damodaran, 1991; Bryant & McClements, 1998). Ca^{2+} may also induce aggregation because of their ability to act as bridges between the negative charged carboxylic groups on neighbouring whey protein molecules (Hongsparabhas & Barbut, 1997b). Much lower concentrations of divalent ions are needed to cause aggregation and gelation than monovalent ions because they are much more effective at shielding electrostatic interactions and because of their ability to form salt bridges.

The process of heat-denatured WPC powder in the Edgecumbe pilot plant was a closely mimic commercial WPC process. The additional processing steps, e.g. dilution, heating, pH adjusting, can be easily incorporated into the current plant. In the current WPC plant at Edgecumbe, there are three UF lines. Two of them are main UF lines for daily production of normal WPC products, while the third line is used for specific WPC products (e.g. low lactose WPCs). These UF lines could be used to produce heat-denatured WPC products. It only needs to set up heating and cooling system after the first two UF lines. The 1° retentate from the first two UF lines could be heated under certain conditions before the second UF, which can be carried out in the third UF line.

CHAPTER 7
CONCLUSIONS AND RECOMMENDATIONS

The present study consisted of three parts. The preliminary study, carried out in the laboratory, investigated the effects of heat treatment, protein concentration and pH adjustment on denaturation and aggregation of whey proteins in the UF retentate, in order to identify suitable conditions for producing heat-denatured WPC containing "soluble" aggregates of whey proteins. The second part involved the production of heat-denatured WPC powders in the pilot plant at Massey University and Anchor Products, Edgecumbe using the conditions determined in the preliminary study. The third part involved characterisation of the WPC powders and determination of their functional properties.

7.1. Experimental methodology

Native-PAGE was used to determine the loss of individual whey proteins in acid whey, UF retentate and WPC powder during heat treatment. This provided qualitative and quantitative evaluation of denaturation and aggregate formation of whey proteins in these systems. The results revealed considerable effects of protein concentration, pH, heating temperature and time on the loss of native proteins and formation of intermediate aggregates, which play a key role in the functionality of the desired WPC products.

Production of heat-denatured WPC powder was carried out in the pilot plant by adding an additional step of heat treatment prior to UF in the current WPC process (Figure 3.2). Viscosity and gel strength were tested by a rotational viscometer and Instron, respectively. Information on the properties of heat-denatured WPC solutions and gels induced by reheating, addition of GDL, $CaCl_2$ or NaCl at different incubation temperatures was obtained.

In present study, the process for producing denatured WPC powder was developed. Both technical and commercial applications of this process are feasible in the current commercial plants. In order to achieve the desired functionality of WPC products, specific types of aggregates are required, i.e. "soluble" intermediate aggregates. The preliminary experiment established suitable heating conditions

(1% protein solution, pH 7.0 at 80°C for 20 min) for formation of these aggregates under low ionic strength (using UF retentate). The denatured WPC powders were produced by addition of a heating step to the current WPC process. This developed process is easy to incorporate into the current WPC plant. As discussed in Section 6.5, current process for producing specific WPC products (low lactose WPCs) can be modified to produce heat-denatured WPC. This can be simply achieved by setting up a heating and cooling system between the first UF and the second UF lines. Therefore, the cost for producing denatured WPC is expected to be slightly higher than the low lactose WPCs.

Attempts have been made by several researchers to produce denatured WPC products with desired functionality (Palatasa Havea, personal communications). However, these studies were largely unsuccessful. In other studies, whey was used as a raw material. As confirmed by the preliminary study, because of its high ionic strength, whey would form large "insoluble" aggregates during heat treatment. Upon UF, these denatured proteins could form gels when the concentration of the retentate reached a certain level.

According to the present result, the denatured whey protein solution tended to form gel at low temperatures, even without additives. Therefore, it is necessary to control the system temperature at > 50°C during UF process to avoid the gel formation. Moreover, the time for drying of 2° retentate from the UF is critical, which was suggested to be ≤ 4 hrs.

7.2. Functional properties of heat-denatured WPC

Heat-denatured WPC powders produced in the pilot plant at Massey University and Anchor Products had desired functional properties, which were different from the normal WPC powder. The denatured WPC were capable of forming viscous solutions or gels at ambient temperature in the absence or presence of additives. Upon addition of GDL, $CaCl_2$ or NaCl, the viscosity of 10% denatured WPC solutions increased with GDL or salt concentration and gel was formed after incubation at 5-40°C. By contrast, the viscosity of 10% unheated WPC solutions did not change and no gelation occurred under any incubation conditions.

In the absence of additives, the viscosity of denatured WPC increased with protein concentration, incubation time and decreased with incubation temperature. However, upon addition of GDL or $CaCl_2$, the viscosity and gel strength increased with protein concentration and incubation time as well as with temperature.

7.3. Potential applications for heat-denatured WPC

The heat-denatured WPC produced in the pilot plants at Massey University and Anchor Products, Edgecumbe, had desirable properties, namely the ability to gel without heating and form highly viscous solutions. The high viscosity of the solution and the ability to form a gel upon addition of GDL or salt at 5-40°C would enhance their application in food systems, such as in comminuted meat, pressed ham/bacon, mayonnaise and dressing, yoghurt and bakery products.

Heat-denatured WPC has potential application in pressed ham or bacon is because it becomes viscous or starts to gel when salt is added. It will glue the meat pieces together and also bind excess water. During heating it will also gel, resulting in a firm and sliceable texture, meaning less slicing-loss and increased yield.

Commercial WPC has always had some difficulties functioning in comminuted meat products because the optimum functionality demands heating. Heat-denatured WPC is able to give a high viscosity in a cold state (5-40°C), which will perform water binding and texture forming properties. Thomsen (1995) reported that the fat binding properties of whey protein texturiser (WPT), a denatured whey protein product, were superior to conventional proteins like caseinate and soy proteins.

The ability to thicken solutions or form gels at 5°C and 40°C is of particular interest in mayonnaise/dressing and yoghurt products.

7.4. Recommendations for future study

The heat-denatured WPC produced is expected to have different functionality. In addition to viscosity and gelation, much more remained to be investigated to have a clear understanding of factors that influence the properties of the denatured WPC products. Based on the results obtained from the project, the following subjects are recommended for future study.

Process optimisation

The results from our preliminary study showed that whey protein concentration prior to heat treatment and the heating temperature had major effects on denaturation and aggregation of whey proteins. Control of heating process was most important for producing "soluble" intermediate aggregates, which were required for the desired functionality of final products. In the present study, the pilot plant trials did not exactly reproduce the results obtained at a laboratory scale. Under the same heating conditions, less intermediate "soluble" aggregates were formed in the denatured WPC produced in the pilot plant trials, especially at Edgecumbe, compared to that in diluted retentate solutions heated in the laboratory. This was due to lack of heating process control in the pilot plant trials. Further studies in this area need to be carried out.

The current study recommends heating 1% protein solution at 80°C. Heat treatment of large quantity of water can incur high cost during manufacture. Increasing protein concentration before heating could reduce the cost of production. It is, therefore, suggested to consider the combination effects of heating temperature and time on denaturation of whey proteins at higher protein concentration. The ability to produce the desired intermediate aggregates at higher protein concentration would reduce the processing costs significantly.

Processing costs

The costing for producing WPC products is a very important issue in the industry. It is highly recommended to determine the cost for the production of heat-denatured WPC comparing to the cost of original commercial WPC. This exercise was not part of the current study, but it will be beneficial for the industry if the investigation is conducted alongside economic feasibility studies.

Effect of spray drying on protein aggregates

In addition to the factors discussed in this work, the spray drying during WPC process would have a certain effect on whey protein aggregates formed during heat treatment as suggested by the different gelation ability of pre-denatured WPC from Edgecumbe trials and Massey trials. It is recommended to investigate the effect of

spray drying on the type of protein aggregates as well as the functional properties of WPC manufactured using different parameters of spray drying systems.

Evaluation of microstructure of aggregates and gels

Aggregates of whey proteins formed during preheating play an important role in gelation and other functional properties of heat-denatured WPC. It is desirable to determine the type and size of aggregates as well as the structure of gels formed under various conditions by size exclusion chromatography, scanning electron microscopy, confocal scanning laser microscopy or transmission electron microscopy. It was clear from the results of this study that the viscosity and gel strength of heat-denatured WPC were affected by protein concentration, incubation temperature, time and concentrations of GDL, $CaCl_2$ or NaCl added. Under different conditions, the solutions or gels formed by pre-denatured WPC will have different structures. The transformation from one type of structure to another is of importance to the food industry. Besides, the information will also be very useful to choose an optimum protein concentration prior to heating and optimum temperature, as literatures suggested that higher protein concentration and higher preheating temperature lead to harder and clearer gels.

Effect of reheating in the presence of salts on gel properties

As was discussed in Chapter 5, it would be the common case that heating in the presence of salts if heat-denatured WPC used as thickening or gelling agents in food systems. It is, therefore, needed to clarify the effect of reheating on properties of heat-denatured WPC solutions or gels upon addition of $CaCl_2$ and NaCl (as these two salts are commonly used in food systems).

Application of pre-denatured WPC in meat and yoghurt systems

The results demonstrated that heat-denatured WPC was capable of forming high viscous solutions or gels upon addition of salts or acidulant at 5°C, 20°C and 40°C. These properties provide an opportunity to apply the heat-denatured WPC to food products where there is no further heat treatment required. It is recommended to focus on applications of heat-denatured WPC in meat, fish, yoghurt and emulsified

products such as mayonnaise and dressing to know the performance of heat-denatured WPC in terms of cold-set gelation and texture modification in "real" food systems.

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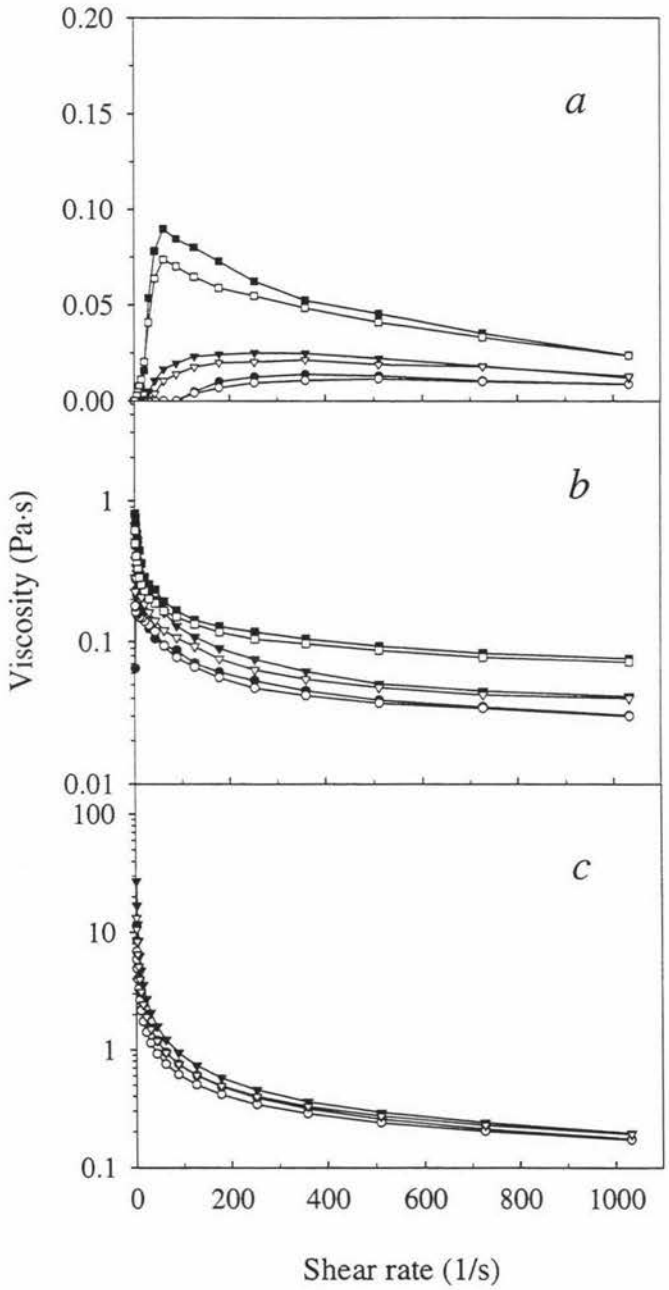
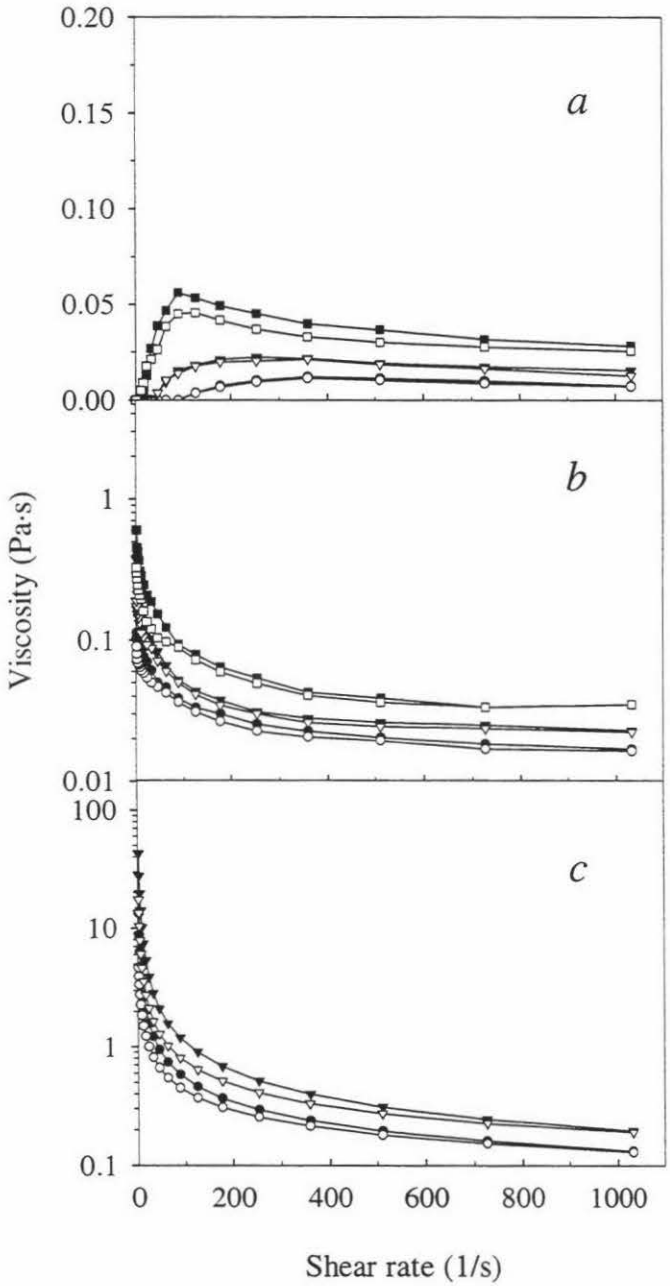
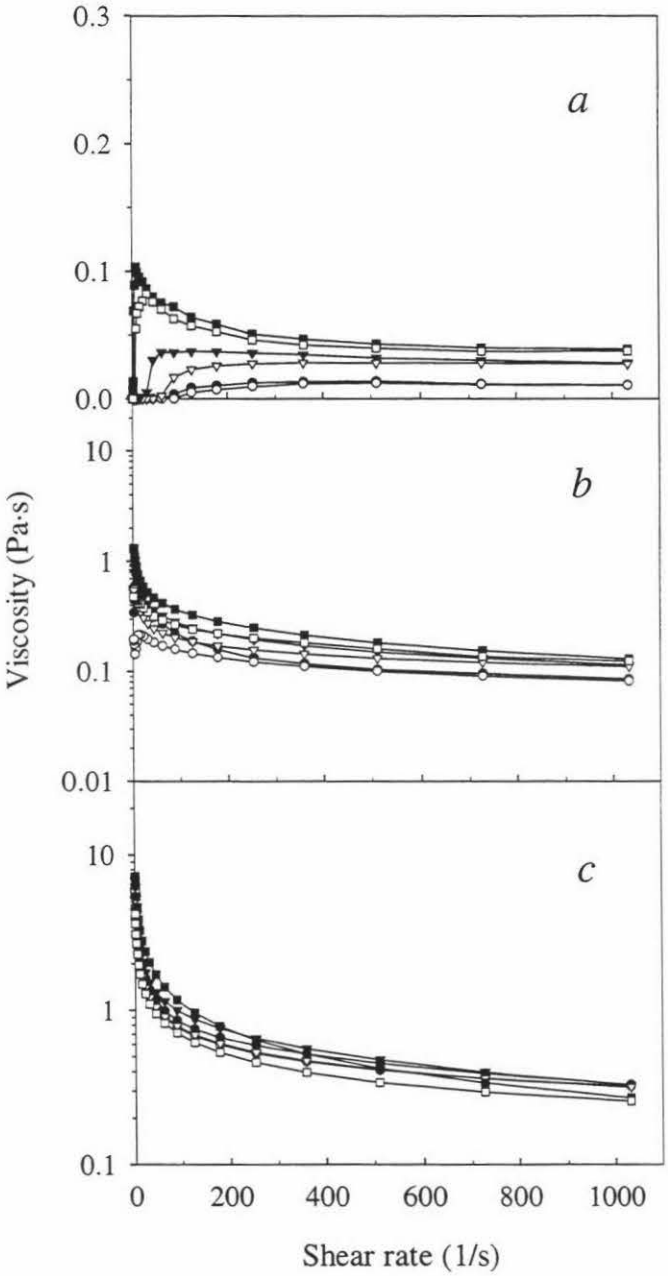


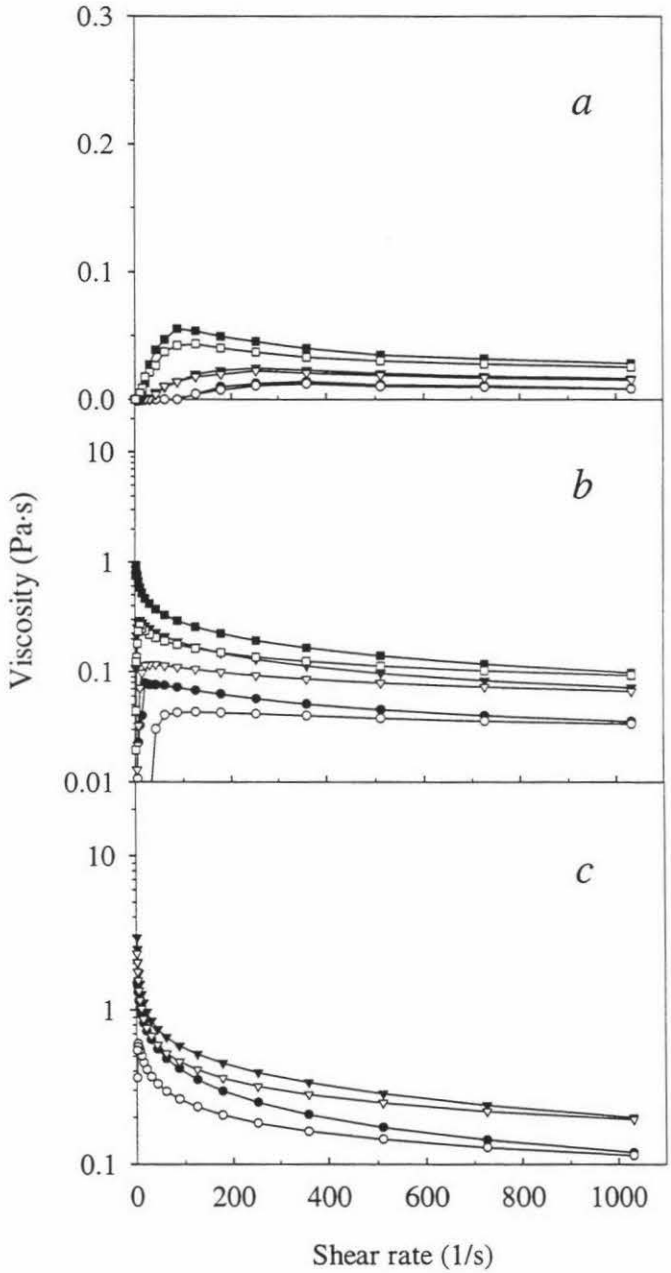
Appendix 5.1. Viscosity of 5% (*a*), 10% (*b*) and 15% (*c*) heat-denatured WPC solutions (H0913) incubated at 20°C for 1 h (● ○), 5 h (▼ ▽) or 24 h (■ □) as a function of shear rate. Shear rate increased from 0 to 1032.5 sec^{-1} (800 rpm) (● ▼ ■) and then decreased from 1032.5 to 0 sec^{-1} (○ ▽ □) in a step manner.



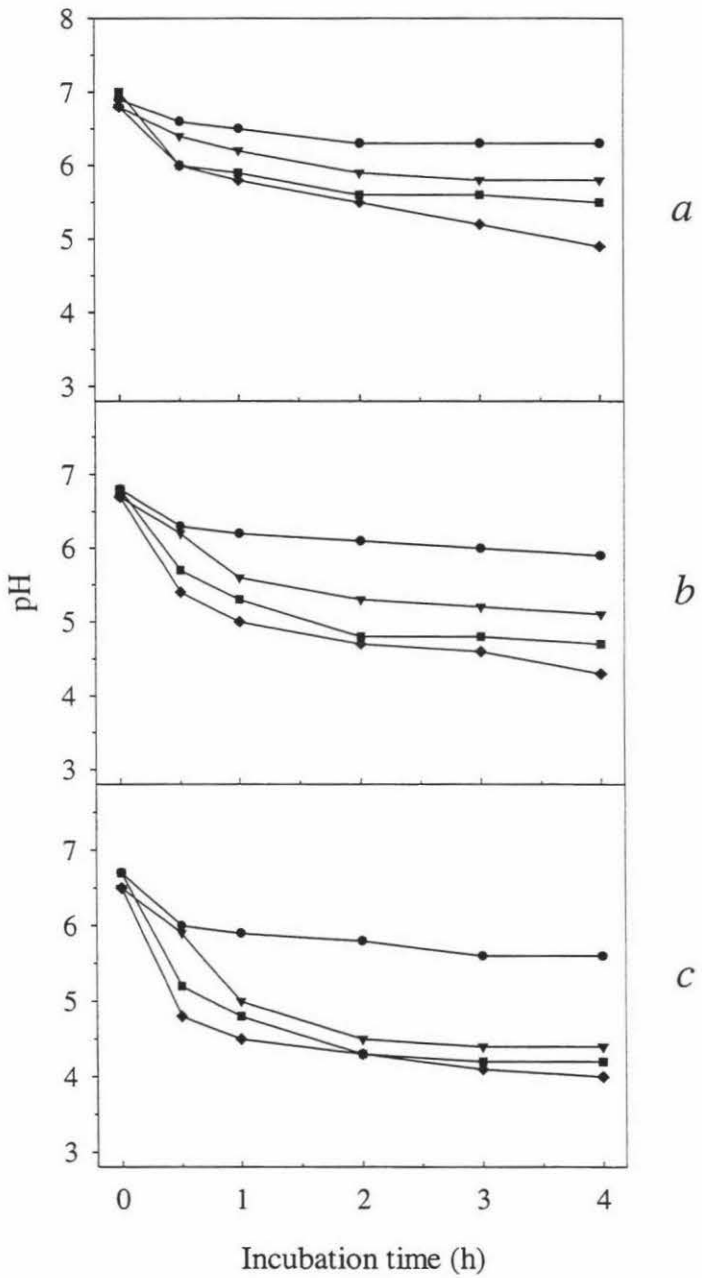
Appendix 5.2. Viscosity of 5% (*a*), 10% (*b*) and 15% (*c*) heat-denatured WPC solutions (H0913) incubated at 40°C for 1 h (● ○), 5 h (▼ ▽) and 24 h (■ □) as a function of shear rate. Shear rate increased from 0 to 1032.5 sec^{-1} (800 rpm) (● ▼ ■) and then decreased from 1032.5 to 0 sec^{-1} (○ ▽ □) in a step manner. The 15% WPC formed gel after incubation for 24 hrs.



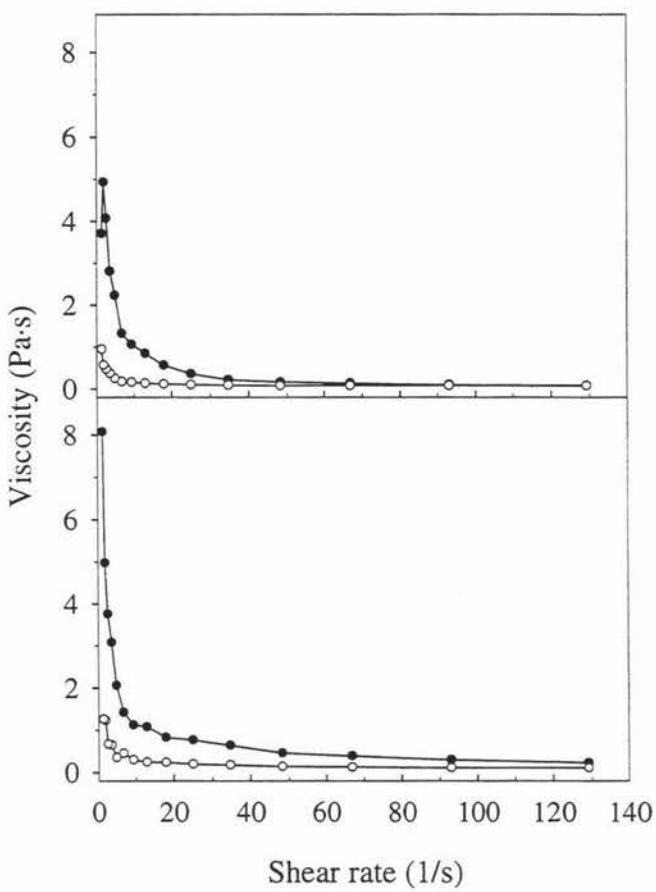
Appendix 6.1. Viscosity of 5% (*a*), 10% (*b*) and 12% (*c*) heat-denatured WPC solutions (H1015) incubated at 20°C for 1 h (● ○), 5 h (▼ ▽) and 24 h (■ □) as a function of shear rate. Shear rate increased from 0 to 1032.5 sec^{-1} (800 rpm) (● ▼ ■) and then decreased from 1032.5 to 0 sec^{-1} (○ ▽ □) in a step manner.



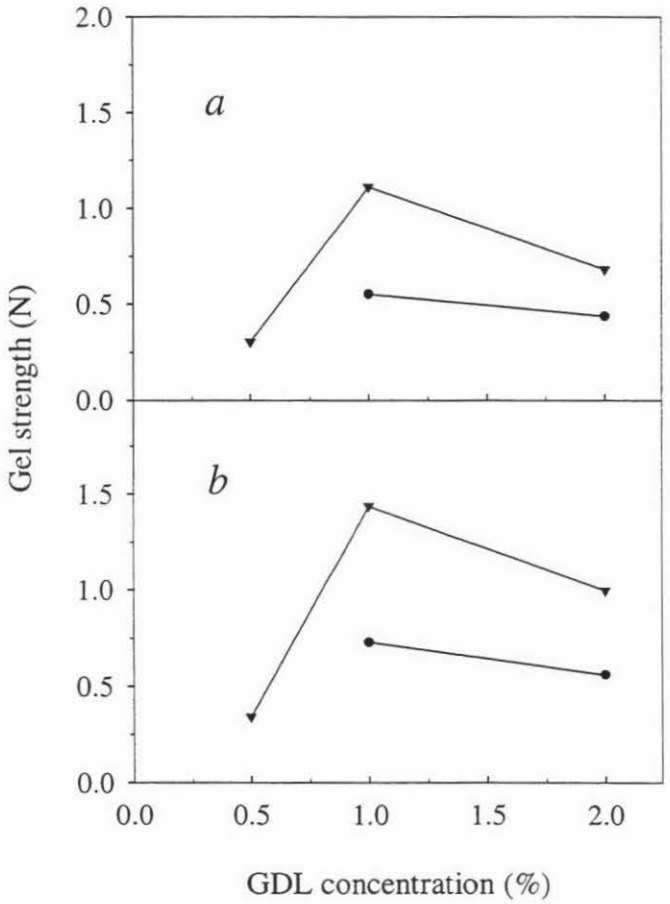
Appendix 6.2. Viscosity of 5% (*a*), 10% (*b*) and 12% (*c*) heat-denatured WPC solutions (H1015) incubated at 40°C for 1 h (● ○), 5 h (▼ ▽) and 24 h (■ □) as a function of shear rate. Shear rate increased from 0 to 1032.5 sec^{-1} (800 rpm) (● ▼ ■) and then decreased from 1032.5 to 0 sec^{-1} (○ ▽ □) in a step manner.



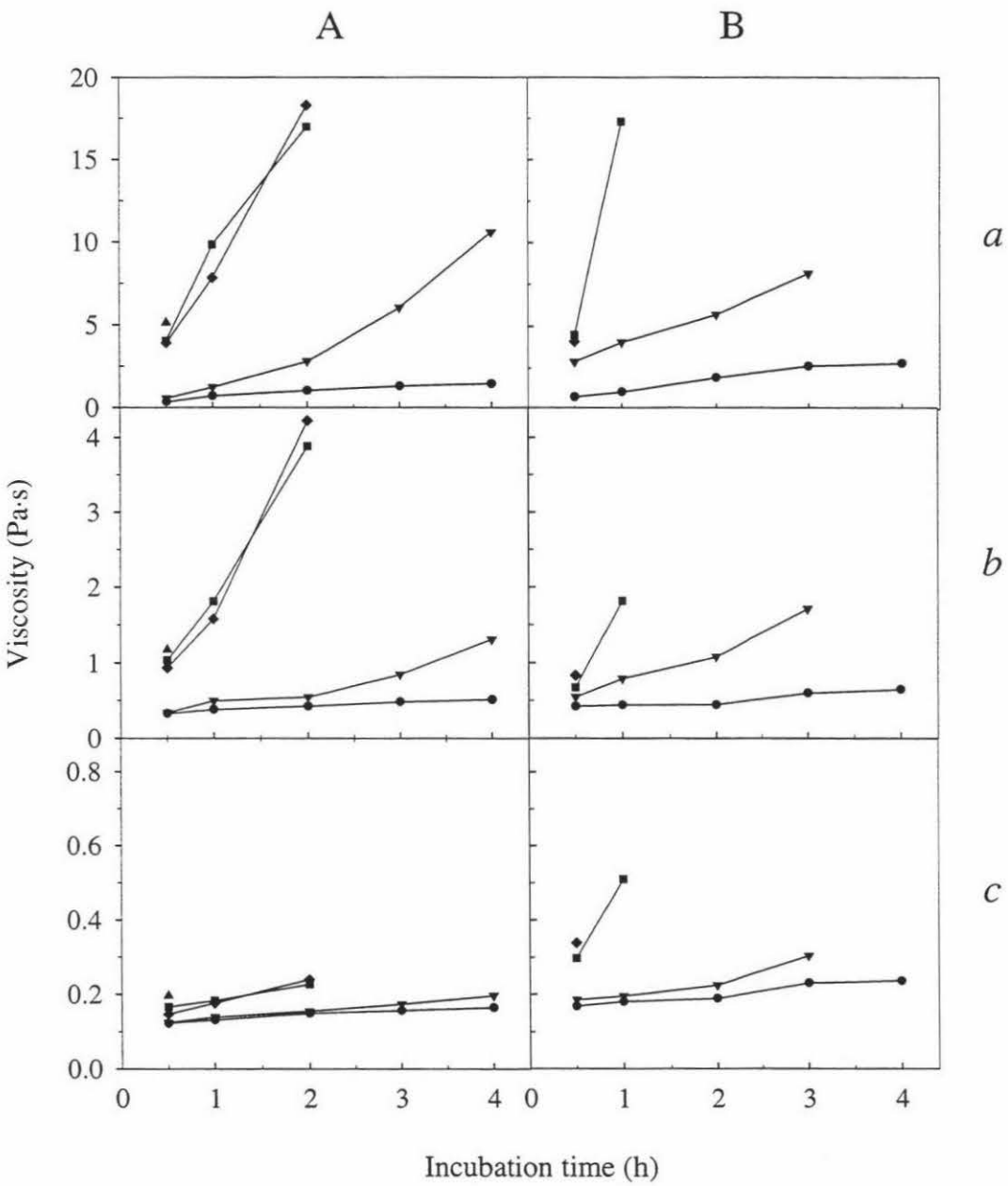
Appendix 6.3. Changes in pH of 10% heat-denatured WPC solutions (H1020) upon addition of 0.5% (*a*), 1% (*b*) and 2% (*c*) GDL at 5°C (●), 20°C (▼), 30°C (■) and 40°C (◆).



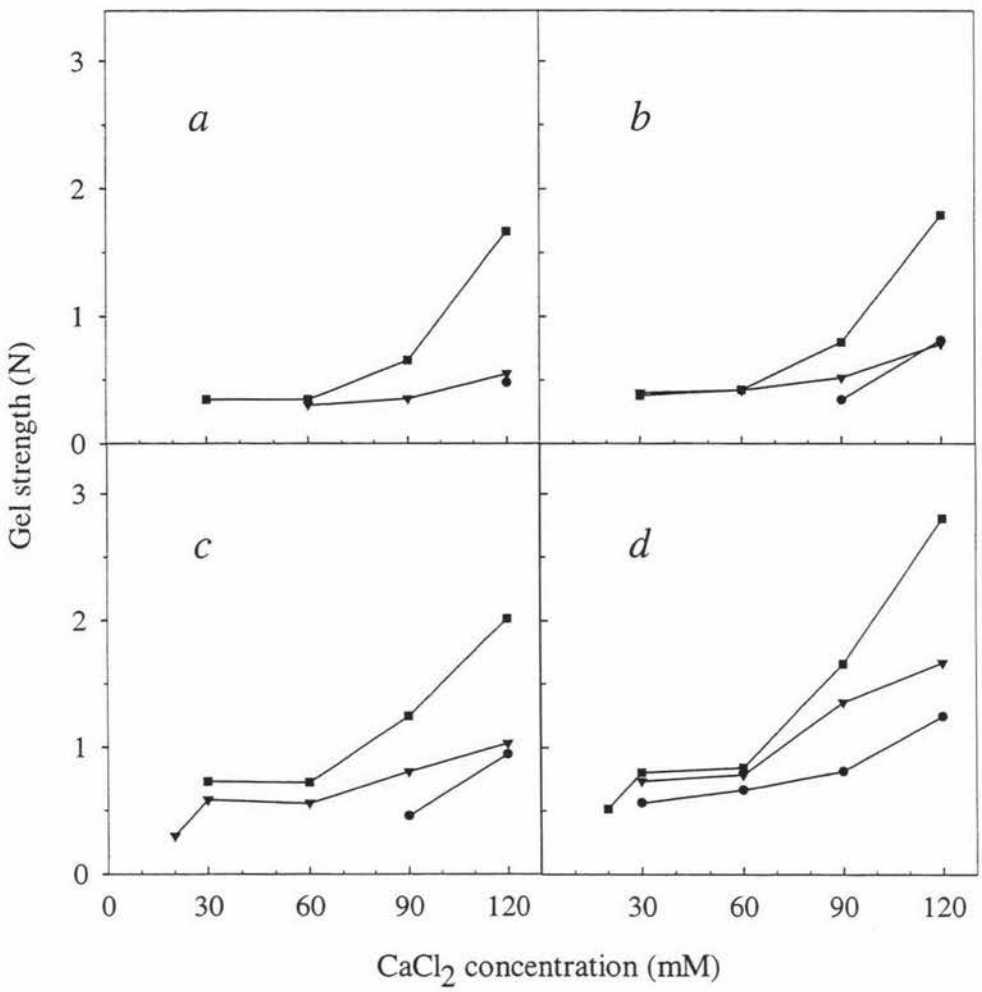
Appendix 6.4. Viscosity of 10% heat-denatured WPC solutions of H1015 (*a*) and H1020 (*b*) upon addition of 1% GDL at 40°C for 1 h as a function of shear rate. Shear rate was increased from 1.29 to 129.1 sec^{-1} (●) and then decreased from 129.1 to 1.29 sec^{-1} (○) in a stepwise manner.



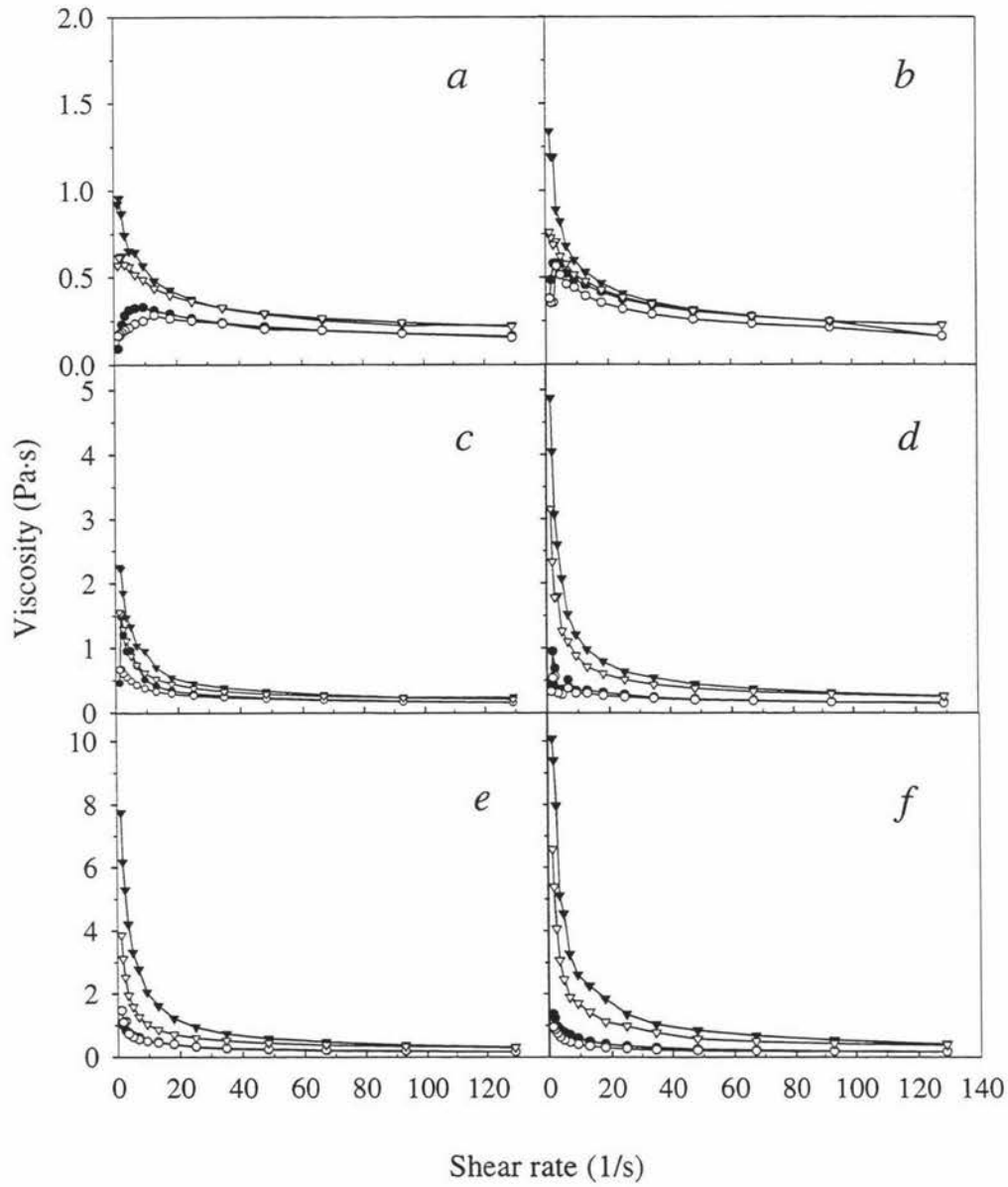
Appendix 6.5. Effect of incubation temperature and GDL concentration on gel strength of 10% heat-denatured WPC solutions of H1015 (*a*) and H1020 (*b*) during 4 h incubation. The incubation temperature was 30°C (●) or 40°C (▼).



Appendix 6.6. Effect of $CaCl_2$ concentration and incubation time on viscosity of 10% heat-denatured WPC solutions of H1015 (A) and H1020 (B) at 20°C as measured at shear rate of 1.29 (*a*), 12.91 (*b*) and 129.1 (*c*) sec^{-1}. $CaCl_2$ concentration was added at 10 mM (●), 20 mM (▼) 30 mM (■), 60 mM (◆) and 90 mM (▲).



Appendix 6.7. Effect of incubation temperature and $CaCl_2$ concentration on gel strength of 10% heat-denatured WPC solutions (H1020) incubated at 5°C (●), 20°C (▼) and 40°C (■) for 1 h (*a*), 2 h (*b*) 3 h (*c*) and 4 h (*d*).



Appendix 6.8. Viscosity of 10% heat-denatured WPC solutions (H1020) incubated with 20 (*a*), 50 (*b*), 100 (*c*), 200 (*d*), 300 (*e*) and 400 (*f*) mM NaCl at 20°C for 1 h (● ○) and 4 h (▼ ▽) as related to shear rate. Shear rate increased from 1.29 to 129.1 sec^{-1} (● ▼) and then decreased from 129.1 to 1.29 sec^{-1} (○ ▽) in a stepwise manner.