THE EFFECT OF MICRO- AND MACRO-MOLECULES ON THE MICROSTRUCTURE AND GEL CHARACTERISTICS OF WHEY PROTEIN CONCENTRATE AND ALBUMEN.

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The candidate confirms that the work submitted is his own and that appropriate credit has been given where reference has been made to the work of others.

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Abstract

Avian egg albumen and whey protein concentrates from milk are widely used in the food industry as binder systems, emulsifiers, and foaming agents and for general consumption. In meat-like analogues such as Quorn, the whey protein concentrate and egg albumen are relied upon to produce tough gels with the application of heat. Like many food products, the heat-induced gelation of the protein molecules depend on the environmental conditions and other materials present in the system as well as any interactions between them. A study of a range of ingredients as well as environmental the factors of pH, material concentration and various blends of whey protein concentrate and albumen was initiated in the hope of quantifying the effect of the materials on gel structure. Texture Profile Analysis (TPA), stress relaxation, protein gel dissolution, colour measurement and confocal laser scanning microscopy (CLSM) were employed to assess, measure and quantify the relationships between the environmental factors and the added materials.

The results obtained indicated that whey protein and albumen form an interpenetrating gel under normal conditions. The optimum ratio at which the combined binder exhibited it maximum values was of the order of 2:1 (whey/albumen). The pH of the media had the biggest effect on the gel properties. Alteration in pH close to the isoelectric point changes the gel from a fine-stranded network to one with a particulate or filamentous network. Significant interactions were observed between all the main variables on at least one of the responses. Addition of hydrocolloids with large molecules in relation to the protein such as methylcellulose and pectin led to phase separation. Methylcellulose induced a change in the gel from one that imbibed water to one that exuded water as the concentration of the material was increased up to 2%. With high methoxyl pectin, there was phase separation at pectin concentration of as little as 0.5% and phase inversion at pectin concentration in excess of 0.5%. The starch products generally delivered the largest increase in the gel hardness, but there were changes to other gel properties depending on what type of starch was used. Milk-derived ingredients such as lactose, casein and glycomacropeptide (GMP) were not necessarily compatible with a whey protein/albumen gel.

Dissolutions tests with protein perturbing agents such as DTE, SDS and urea showed that some of the added materials interfered with protein-protein gel formation by one of two ways (1) by blocking the formation of the bonds necessary to stabilize the protein structure and/or (2) interacting with the water molecules in preference to the protein molecules. In addition the CLSM micrographs proved that there was indeed phase separation of the molecules when the conditions were not favourable.

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ABBREVIATIONS

CLSM	Confocal Laser Scanning Microscope(y)
DNA	De-oxyribonucleic acid
DTE	Dithioerythritol
EDTA	Ethylene diaminetetraacetic disodium salt
GMP	Glycomacropeptide
HCl	Hydrochloric acid
kDA	kilo Dalton
mRNA	messenger Ribonucleic acid
NaOH	Sodium Hydroxide
RNA	Ribonucleic acid
SDS	Sodium Dodecyl Sulphate
SH	Sulphydryl
SS	Disulphide
TPA	Texture Profile Analysis
tRNA	transfer Ribonucleic acid
UV	Ultra Violet
WHC	Water Holding Capacity
w/v	weight/volume

CHAPTER 1

INTRODUCTION

1.1 QUORN™ MEAT-FREE PRODUCTS

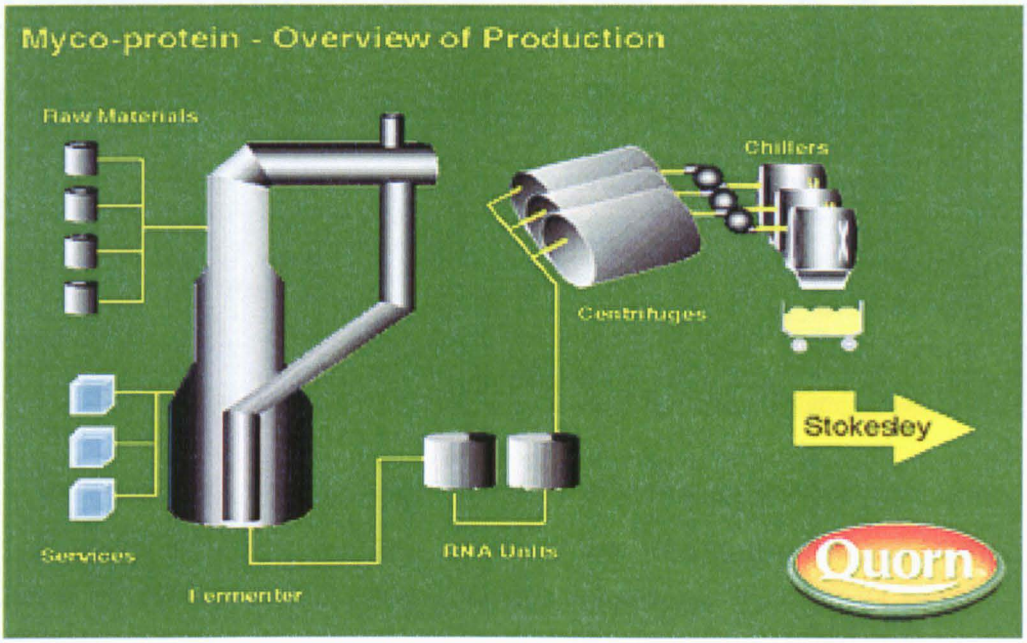
In the 1950s, it was predicted that by the 1980s, there would be a world shortage of good quality protein. In response to this perceived threat, Lord Rank, of Rank Hovis McDougal (RHM), commissioned research into using starch products as a substrate for the conversion of single celled organisms into a high protein product suitable for human consumption. Following an extensive screening process, the filamentous fungus *Fusarium venenatum*, discovered in 1967, was isolated as the best candidate. Mycoprotein refers to the protein mass-produced from the mycelia of the fungus *Fusarium spp.* Although originally conceived as a protein-rich food supplement for the predicted global famine, the food shortage never materialised. In 1980, RHM was given permission to sell mycoprotein for human consumption after a ten-year evaluation programme. In 1989, it was observed that almost half of the UK population was reducing their intake of red meats and a fifth of young people were vegetarians. As a result, Marlow Foods (set up by RHM) decided to market and sell *Quorn* as a new healthy meat analogue, which was free of animal fats and cholesterol. *Quorn* refers to the brand name for the range of foods sold by Marlow Foods containing mycoprotein. *Quorn* products are sold mostly in Western Europe and consist of a range of meat-like cooking ingredients and ready meals.

Mycoprotein is manufactured by growing the fungus in continually oxygenated water in large, sterile fermentation tanks. During the growth phase glucose is added as a food for the fungus, as are various vitamins and minerals to optimise the growth of the organism. After a period of time of continuous growth, the fungus cells are harvested as a 'broth'. Since the purine bases in nucleic acids are metabolized to

uric acid, an excess in the blood of which can give rise to gout, the ribonucleic acid (RNA) content of mycoprotein is reduced from 10% to less than 2% (dry weight) by rapidly heating the "broth". The heat process causes complete loss of cell viability and a leaching of most of the cell RNA into the supernatant. Following RNA reduction, the mycelia are recovered by a centrifugation dewatering process to produce a thick and viscous paste similar to bread dough. At this point, the paste that is collected has a water content of approximately 75%, and is denoted by the name mycoprotein.

Figure 1.1-A

Overview of the Production of Mycoprotein

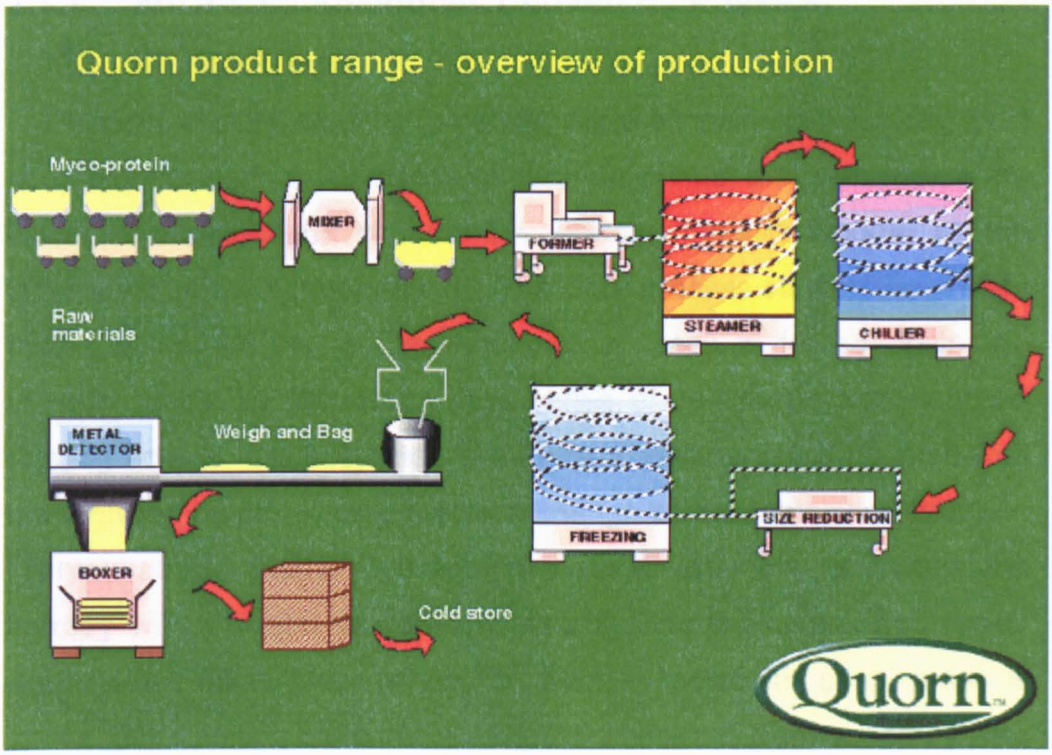


To make *Quorn* products, the mycoprotein mass is mixed with whey protein concentrate and/or avian egg albumen among other things, which act as a binder. It is then textured, giving it some of the grained character of meat, and processed further to yield mince, chunks etc. The texture is made possible mostly by the filamentous nature and the size and shape of the hyphae. In the texturized format, *Quorn* products have a varying colour and a mild flavour resembling the imitated meat product, and are suitable for use as a replacement for meat in many dishes, such as stews and casseroles. The final *Quorn* product is high

in vegetable protein and dietary fibre and is low in saturated fat and salt. The amount of dietary iron it contains is lower than that of most meat products.

Figure 1.1- B

Overview of the Manufacture of Quorn Products



Over the last the last ten years, the consumption of meat-free products has grown by almost 500%. (Meat-free in the context of this thesis refers to non-meat products that have been formulated to have mouth-feel and taste similar to meat. It does not include vegetable-based products or pulses). By the end of 2006, it was estimated that the meat-free market was worth £156m and is estimated to grow to about £192m by 2011 (Mintel, 2006). Most of these products are consumed as part of a main meal. Changes in eating patterns mean that many people, whilst cutting down on the amount of meat consumed, especially red meat, are increasingly demanding meat-free products that still taste like and have the texture of meat.

Quite a few different types of meat-free products are available in the UK and include those sold under the *Cauldron*™, *Linda McCartney*™, and *Tivall*™ brands. Many of the meat-free products are made using

protein derived from plants as the raw material. The two main techniques by which meat-type analogues are made are by

- manipulation of the proteins by physical means
- manipulation of the proteins by the presence of other molecules.

1.1.1 Meat-Like Analogues Derived By Physical Manipulation

Physical manipulation is generally done by extrusion cooking of a soup of proteins. It involves the application of heat and pressure in the barrel of an extrusion cooker. The protein mass is worked by the screws within the extruder barrel. The work process causes the protein matrix to unfold. As the 'molten' protein mass is forced down the extruder barrel by the action of a series of co-rotating and counter-rotating screws, it is subjected to intense pressure, which coupled with the high temperature, effectuates a change to the molecular structure (Yuryev *et al.*, 1991). Due to the 'natural' attempts to reduce the entropy with the aim of attaining stability, the molecules aggregate. Aggregation takes place when the molten 'molecular soup' mass is forced into a setting zone of the extruder where it is transported by plug flow. At this time, the protein molecules re-align, the gentle flow leading to striations similar to the myofibril bundles found in muscle.

The texture of meat analogues produced by extrusion cooking of protein tends to be one-dimensional. The use of other ingredients in addition to protein to provide texture not only is easier but also offers more chance for deliberate manipulation of gel characteristics at a molecular level.

1.1.2 Meat-Like Analogues Derived By Ingredient Manipulation

In the molecular approach method, reliance is placed on the functional characteristics, especially gelation and water-holding capacity (WHC), of the protein molecules to provide texture. Proteins such as egg albumen and whey protein will gel with the application of heat. Successful utilisation of these proteins as functional ingredients

requires a basic understanding that allows adjusting the mechanical properties of the resulting gel to yield a variety of textures. Gel mechanical strength and elastic modulus are directly affected by the concentration, conformation and type of molecules present.

Mycoprotein is not a true globular protein in the manner of soya, wheat, egg and milk proteins but is a mass of inert cells of the *Fusarium* organism. It has a mycelium of narrow, branched and septate hyphae. This basis for a naturally fibrous (and therefore 'chewy') texture has been exploited in the formulation of meat analogues. In reality, mycoprotein can be described as an open-ended 'tube' of protein mass protected by the cellulose-based cell wall of the organism.

The texture of many of these meat-free products is dependent on (1) the manner in which discrete particles are held within a continuous phase and (2) the ratio of discrete phase to continuous phase. The gel formed by the heat-induced gelation of proteins forms the continuous phase whilst the mycoprotein fibres constitute the discrete phase. For mycoprotein-based products, the characteristic meat-like texture arises from the 'fibre-gel' composite that is produced and not just the structure of the gel.

1.2 Proteins

Proteins are complex, high molecular weight organic compounds that consist of amino acids joined by peptide bonds (Wu *et al.*, 2007). Because of their dynamic structure and amphiphilic nature, they possess varying functional properties (Frejj-Larson *et al.*, 1996). In effect, it can be said that they are amino acid chains that are folded into unique 3-dimensional structures.

Protein synthesis is the multi-step process by which cells build proteins. It begins with amino acid synthesis followed by transcription, translation and then folding.

1.2.1 Amino acid synthesis

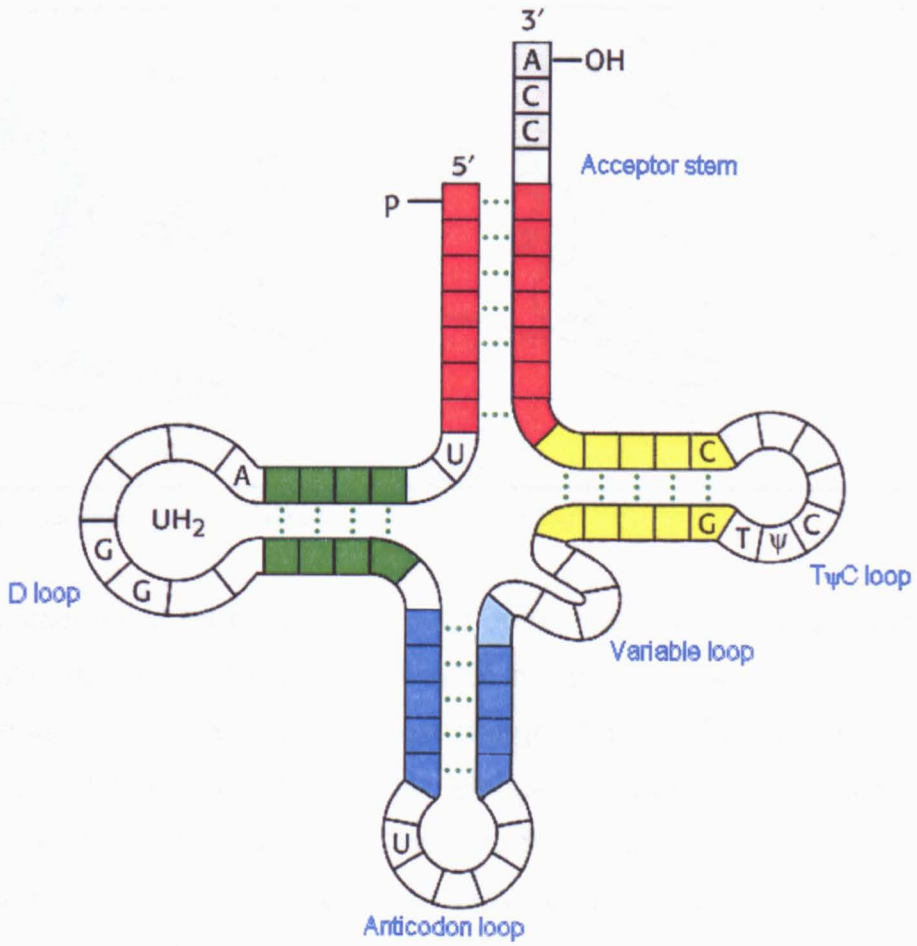
Amino acids are the building blocks for the polypeptides known as proteins. Amino acid synthesis is the set of biochemical processes or metabolic pathways within the cell that build the amino acids from carbon sources like glucose.

1.2.2 Activation

The amino acids are loaded onto transfer-RNA molecules and transported to the ribosome for use in the process of translation. The amino acid is joined by its carboxyl group to the 3' OH of the transfer-RNA by esterification. At this point the transfer-RNA is said to be 'charged' and the process is known as activation.

Figure 1.2-A

The Structure of Transfer-RNA



Adapted from http://www.med.unibs.it/~marchesi/protsyn.html

1.2.3 Transcription

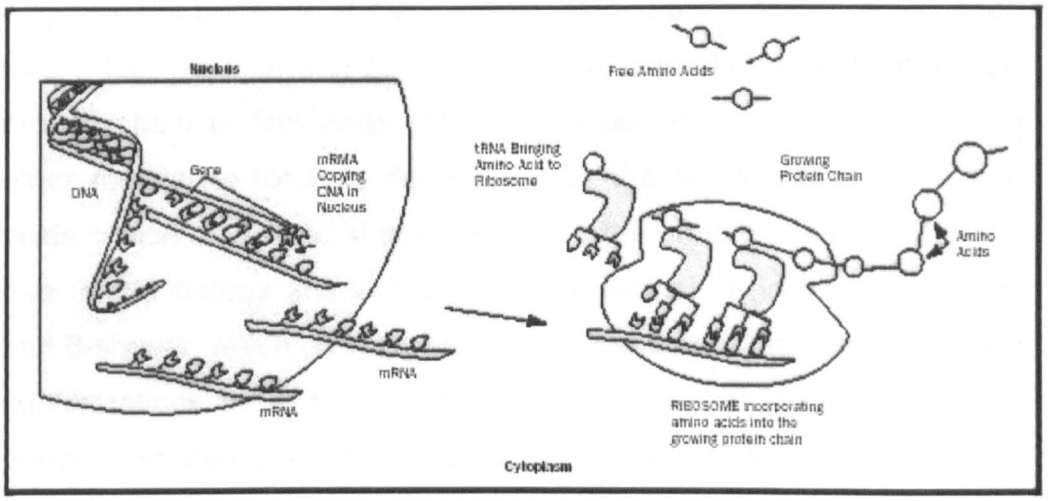
Transcription occurs in the nucleus. It is the process by which the DNA helix is unravelled. Messenger-RNA from the DNA nucleus is decoded to produce a specific polypeptide according to the rules specified by the genetic code. It is then transferred out of the nucleus to the cytoplasm where the ribosomes are located.

1.2.4 Translation

Translation proceeds in the ribosomes in an orderly manner. When the protein is "translated" from messenger-RNA, it is created from N-terminus end to C-terminus end. Translation proceeds in phases: initiation, elongation and termination.

Figure 1.2-B

Protein Synthesis From mRNA and tRNA



Adapted from www.chemistryexplained.com/images/chfa_04_img

Initiation involves the small subunit of the ribosome binding to the 5' end of messenger-RNA with the help of initiation factors.

Elongation occurs when the next charged transfer-RNA in line binds to the ribosome along with an elongation factor. The ribosome continues to read codons from the 5' to the 3' end. The amino end of the amino acid on a charged transfer-RNA attaches to the carboxyl end of the budding chain during the elongation stage. Then the ribosome

moves to the next codon. The empty transfer-RNA is ejected and the peptidyl transfer-RNA is moved from the A site to the P site.

Termination of the polypeptide happens when the A site of the ribosome faces a stop codon, at which point the polypeptide chain is released.

After translation, modification of amino acids extends the range of functions of the protein. These modifications act on individual residues either by cleavage at specific points, deletions, additions or having the side chains converted or modified. Some of these include acetylation, phosphorylation, methylation, glycosylation, sulphation, deamidation etc. or making structural changes, like the formation of disulfide bridges.

1.2.5 Protein Folding

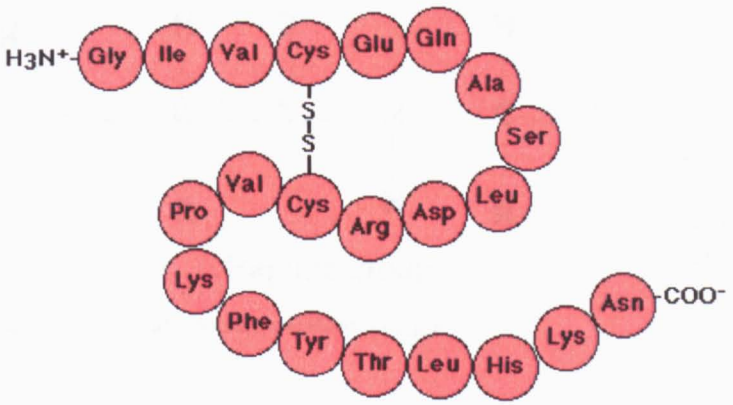
Initially proteins are long extended molecules. However, they soon begin to fold because of the many different type of forces acting upon them. The main driving force is for the hydrophobic portions of the protein chain to fold away from the outside water environment. The other motivating force is determined by the sequence of the amino acids, which drives the shape into which the protein is naturally folded due to the energy and entropy considerations. It includes α-helices and β-sheets, which derive from the stabilisation of the particular chain conformations by hydrogen bonds between the amide groups of the polypeptide chains. It is known as its native state. The actual structures of the proteins themselves are determined and maintained by interactions between the different amino acids that form the polypeptide (Dalgleish & Hunt, 1995). There are four distinct aspects of the structure of a protein.

1.2.5.1 Primary Structure

The protein primary structure is a chain of amino acids and is the first stage after translation. It refers to the exact chemical composition of the biopolymer and to the sequence in which its sub-units are arranged. The primary structure of a biological polymer largely determines the three-dimensional shape that the molecule will assume *in vivo*. The primary structure is almost always stabilized by peptide bonds.

Figure 1.2-C

Lysozyme: An Example of Primary Structure



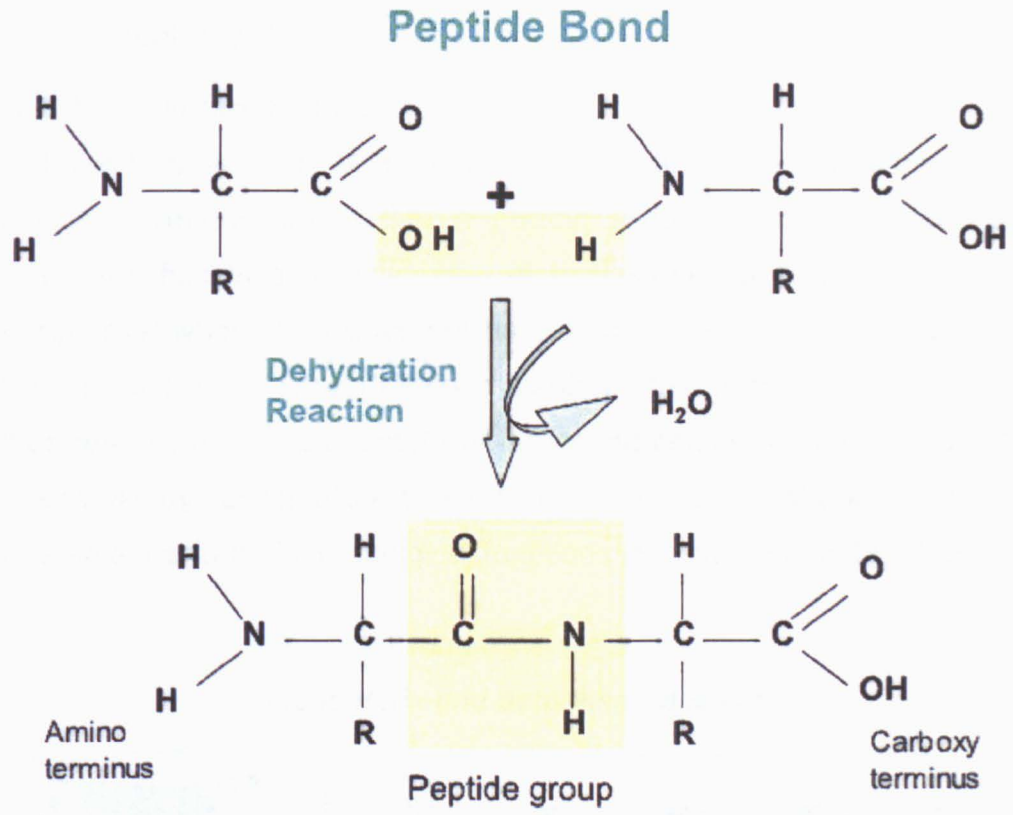
Adapted from users.rcn.com/.../BiologyPages/P/Peptide.gif

1.2.5.1.1 Peptide bonds

A peptide bond is a chemical bond formed between two molecules. It usually occurs between amino acids and is a dehydration synthesis reaction: the carboxyl group of one molecule reacts with the amino group of the other molecule, releasing a molecule of water (Fig.1.2-D). The resultant C-N bond is called a peptide bond and the ensuing molecule is called an amide. Amides can participate in hydrogen bonding as hydrogen bond acceptors or donors.

Figure 1.2-D

Formation of the Peptide Bond



Adapted from www.langara.bc.ca/.../Assets/peptidebond.jpg

1.2.5.2 Secondary Structure

The secondary structure generally reflects how individual molecules in the biopolymer are connected to each other. It does not, necessarily refer to their actual position in the three-dimensional space; the actual positions are considered tertiary structure. It is the regular arrangement or specific geometric shape of the polypeptide chain to stabilise the primary structure by hydrogen bonding between the amine and carboxyl groups. The formation of these secondary structures represents the manner in which the local entropy is decreased (Bryant and McClements, 1998). The stabilisation is partially dependent on the primary structure of the amino acid sequence, Secondary structures are locally defined, meaning that there can be many different secondary motifs present in one single protein molecule. There are two possible types of secondary structure: an alpha helix and a beta sheet. In the case of an alpha helix, the hydrogen bonding causes the

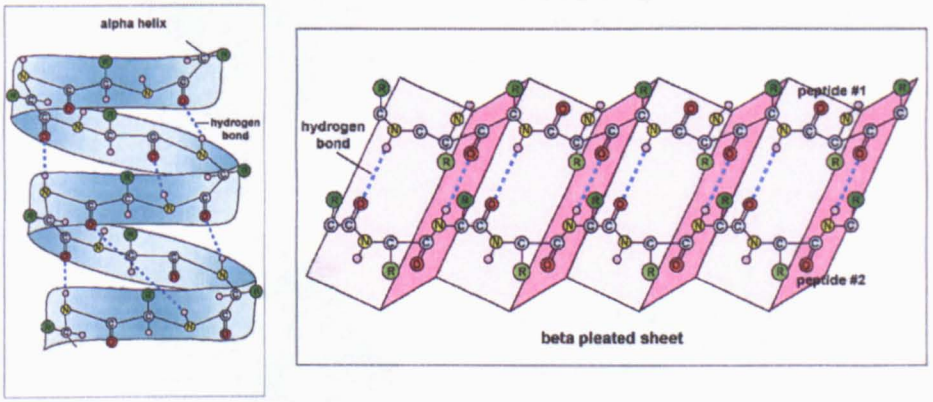
polypeptide to twist into a helix. With a beta sheet, the hydrogen bonding enables the polypeptide to fold back and forth upon itself like a pleated sheet (Fig. 1.2-E).

1.2.5.3 Tertiary Structure

The tertiary structure of a protein is its overall shape and is the final specific geometric shape that a protein assumes. In a solution, a protein will fold in a specific way as to assume the lowest possible energy level where by it does not have to put in energy to maintain its structure and this is what defines a stable protein In addition, although all protein molecules are simple un-branched chains of amino acids, it is generally by coiling into a specific three-dimensional shape that they are able to perform their biological functions (Minetti & Remeta, 2006).

Figure 1.2-E

Alpha Helix and Beta Pleated Sheet

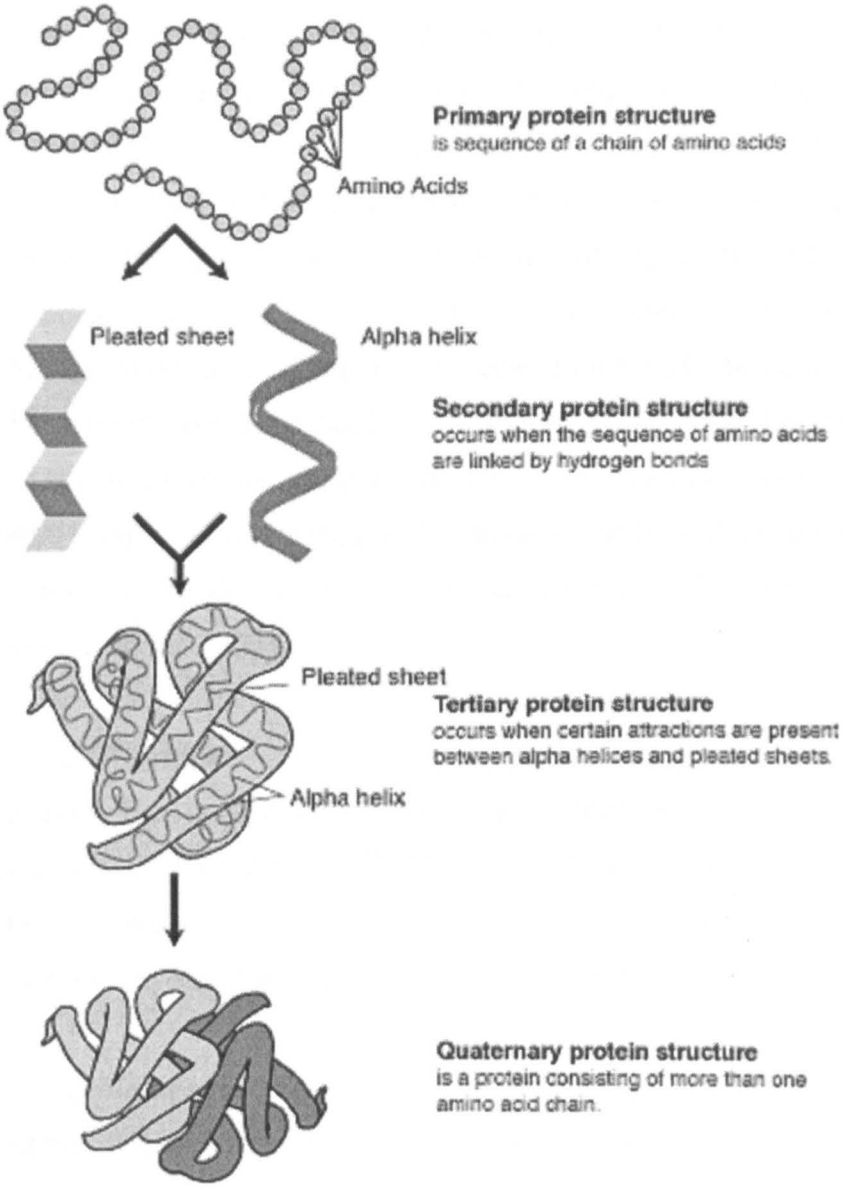


Adapted from student.ccbcmd.edu/.../images/betasheet.jpg

An important type of chemical bond involved in stabilizing the tertiary structure of many proteins is the disulphide bond (See Section 1.2.5.3.3). The final shape is determined by a variety of bonding interactions between the "side chains" on the amino acids. These bonding interactions may be stronger than the hydrogen bonds between amide groups holding the helical structure. As a result, bonding interactions between "side chains" may cause a number of folds, bends, and loops in the protein chain (Higman & Greene, 2006). Invariably, the tertiary structure involves a combination of attractions between the alpha helices and the beta sheets.

Figure 1.2-F

Sequence of Protein Folding



Adapted from content.answers.com/main/content/wp/en/thumb/...

There are several types of bonding interactions between "side chains" and include hydrogen bonding, salt bridges, covalent bonds and non-polar hydrophobic interactions (Sadeghi *et al.,* 2006).

1.2.5.3.1 Hydrogen bonding

The attraction of the partially positive end of one highly polar molecule for the partially negative end of another highly polar molecule is called a hydrogen bond. Hydrogen bonding occurs when a hydrogen atom is covalently bound to a small highly electronegative atom such

as nitrogen, oxygen, or fluorine. The result is a dipolar molecule. The hydrogen atom has a partial positive charge and will interact with another highly electronegative atom in an adjacent molecule particularly hydrogen, oxygen and fluorine. This results in a stabilizing interaction that binds the two molecules together. The hydrogen bond is therefore defined as a type of attractive inter-molecular force that exists between two partial electric charges of opposite polarity. Although stronger than most other inter-molecular forces, the hydrogen bond is much weaker than either of the ionic bond and the covalent bond. Within macromolecules such as proteins, it can exist between two parts of the same molecule, and figures as an important constraint on the overall shape of the molecule. Secondary and tertiary structures in proteins are often held together by hydrogen bonds (Whitfield *et al.*, 2005; Fitzkee & Rose, 2005).

1.2.5.3.2 Salt bridges

A salt or ion bridge is a specific type of weak electrostatic interaction in which a cation such as Na^{+}, Ca^{2+}, Mg^{2+} or Al^{3+} simultaneously binds to the surface of two molecules, each of which has an opposite charge to the ion. It helps to stabilize the structure of the protein.

1.2.5.3.3 Covalent bonding

Covalent bonding is a form of chemical bonding characterized by the sharing of one or more pairs of electrons by two atoms, in order to produce a very strong attractive force, which holds the resultant molecule together. Atoms tend to share electrons in such a way that their outer electron shells are filled.

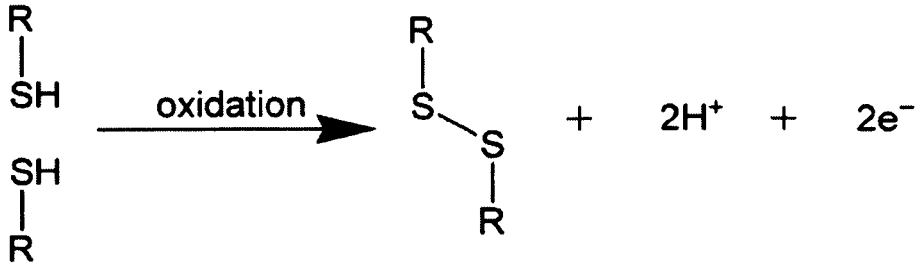
The commonest type of covalent bond in proteins that contain sulphur is the disulphide bond. Disulphide bonds are very important in the folding, structure and conformation of the protein. When two amino acids bind to each other through their side chains, they normally do so through a disulphide bond (Schmidt, 1981). Disulphide bonds are formed principally by a thiol-disulphide reaction (Fig. 1.2-G). The particular side chain involved is the thiol group (-SH). Oxidation of the

thiol group yields a disulphide (S-S) bond. The presence of the disulphide bond is one of the main ways in which the tertiary structure of the protein maintained. Free thiol groups can also participate in thiol-disulphide interchanges with disulphide bonds and often occur under alkaline conditions.

The free thiol and disulphide groups in a native whey protein are located in the interior of the folded molecule and as such are generally unavailable for reactions (Cory & McClements, 1998).

Figure 1.2-G

Formation of Disulphide Bond



1.2.5.3.4 Hydrophobic interactions

In this type of 'bond', water-repelling side chains group together and exclude water molecules. A hydrophobic molecule cannot establish hydrogen bonds with water, thus limiting the "freedom" of the water molecule for switching hydrogen bonds, which makes the whole area around the hydrophobic molecule energetically adverse. As one large area of this kind is energetically more auspicious than two smaller ones, thermodynamics favour hydrophobic molecules clustering together, even though hydrophobic molecules are not necessarily attracted to each other. Hydrophobic interactions are common in establishing the tertiary structure (Lau *et al.*, 1984). The non-polar groups mutually repel water and other polar groups and results in a net attraction of the non-polar groups to each other. Overall, the hydrophobic interaction is a repulsive force. One of the characteristics of hydrophobic interactions is that they get stronger as the temperature is raised up to about 60-70°C after which they slowly begin to lose their strength (Cory & McClements, 1998).

1.2.5.3.5 Ionic or electrostatic interactions

These are interactions that occur between ions or dipoles. They can be either attractive or repelling: like charges repel, while opposite charges attract. These bonds form when the electro-negativities between two atoms are large enough that one 'steals' an electron from the other. The now oppositely charged ions are attracted to each other.

Electrostatic interactions are particularly sensitive to the ionic strength of the medium. The strength and range of the interactions can be reduced significantly in the presence of electrolytes because of electrostatic screening by the ions. They also generally tend to increase in strength with temperature (Cory & McClements, 1998).

1.2.5.3.6 Van-der-Waals interactions

The term van der Waals force originally referred to all forms of inter-molecular forces; however, in modern usage it tends to refer only to London forces: those forces which arise from induced rather than permanent dipoles. Van der Waals forces involve the attraction between temporarily induced dipoles in non-polar molecules as a result of the constant 'sloshing around' of the electrons in the molecule. This polarization can be induced either by a polar molecule or by the repulsion of negatively charged electron clouds in non-polar molecules. Over a series of interacting pairs of molecules, this will give rise to an attractive force. There is little change in the van der Waals interaction between the folded and unfolded state in solution (Bryant & McClements, 1998).

1.2.5.4 Quaternary Structure

Many proteins are actually assemblies of more than one polypeptide molecule, which in the context of the larger assemblage are known as protein subunits. Sometimes, quaternary structures are referred to as protein-protein interactions. The quaternary protein structure involves the clustering of several individual peptide or protein chains into a final specific shape. A variety of the bonding interactions including hydrogen bonding, salt bridges and disulphide bonds hold the various chains into

a particular geometry. In addition to these levels of structure, proteins may shift between several similar structures in performing their biological function. In the context of these functional rearrangements, these tertiary or quaternary structures are usually referred to as conformations and transitions between them are called conformational changes. In an aqueous environment, the proteins are held in their conformations by a number of different interactions such as previously described.

Table 1.2-1

Summary of Type of Protein Molecular Interactions

Type	Sign	Strength	Range	pH	Ionic Strength	Temperature
Hydrophobic	+	Strong	Long	No	No	Increases
Ionic	-	Vary depending on pH an ionic strength		Yes	Decreases	Increases
H_2 bonds	+	Weak	Short	No	No	Decreases
Van der Waals	+	Weak	Short	No	No	-
Disulphide	+	Very strong	Strong	Short	Yes	No

1.3 Protein Denaturation

Denaturation in proteins is a structural change usually caused by heat, acids, bases, detergents, or certain chemicals such as urea. Proteins denature when they lose their three-dimensional structure and thus their characteristic folded structure. Denaturation of proteins involves the disruption and possible destruction of both the secondary and tertiary structures and the bonds that hold these structures together. It is also possible for protein molecules to exist in the intermediate state of a 'molten globule' where the secondary structure is maintained and the tertiary structure is lost (Ptitsyn, 1992). Since denaturation reactions are not strong enough to break the peptide bonds, the primary structure remains the same after a denaturation process (Cheftel *et al.*, 1985). Shimada & Matsushita (1980) described the heat-induced gelation of a protein-water system as a two-stage process: the unfolding or denaturation of a native protein in an aqueous

solution, which is then followed by an aggregation step of protein-protein interaction, resulting in a three-dimensional structure that forms the final network capable of holding relatively large amounts of water.

A balance between the attractive and repulsive forces governs the heat-induced denaturation (Egelandsdal, 1980). The equilibrium is crucial in stabilizing the three-dimensional network of the resulting gel. (Mangino, 1992; Mleko *et al.*, 1997; Lupano, 2000). If the sum of the attractive forces between the molecules is weaker than the sum of the repulsive forces, then the molecules will generally remain in their native state either as individual molecules or as small aggregates (Kinsella & Whitehead, 1989). Kojima & Nakamura (1985) attributed the repulsive forces to surface changes and the attractive forces to various functional groups exposed by the thermal unfolding of the protein. Denaturation can however disrupt the normal α-helix and β-sheets in a protein and uncoil it into a random shape. By exposing parts of the protein molecule that were previously buried, intermolecular interactions are initiated (Kella & Kinsella, 1988).

The most common observation in the denaturation process is the precipitation or coagulation of the protein. The precipitation can be explained as follows. Because the structure of a denatured protein is loose, hydrophobic parts that were deeply buried in the native protein, will encounter the solvent (water). It is energetically unstable when the hydrophobic parts contact water and as a result, denatured proteins will aggregate with each other in order to prevent the hydrophobic parts from contacting with water. Denatured proteins will usually be precipitated because of their aggregation. Altering the process conditions of temperature and time can change the rates and mechanism of denaturation and aggregation (Schmidt, 1981; Boye *et al.*, 1995).

With whey proteins, configurational entropy is important. At relatively low temperatures less than 65°C, the hydrophobic effect dominates and so the globular state is favoured. At higher temperatures, the unfolded state is preferred (Doi, 1993). However, at normal processing temperatures of less than 100°C, the whey proteins only partially unfold

into a 'molten globule' state and do not necessarily undergo complete unfolding into the random coil configuration. (Bryant & McClements, 1998). In this state the whey proteins tend to retain much of the native structure but with the formation of hydrophobic patches on the surface of the molecule.

Protein denaturation manifests itself in several different forms. In enzymes, this may take the form of loss of catalytic activity due to the inability of substrates to bind to active sites. For egg white and some of the whey proteins, denaturation is often irreversible and takes the form of gel formation.

1.3.1 Gel Formation

In the native state, the balance of the forces between the molecules is such that the attractive forces are not strong enough to overcome the repulsive forces. Therefore, the molecules exist as individual entities or very small aggregates (Bryant & McClements, 1989). If the conditions are changed, it is possible to associate the long polymer chains into a three dimensional continuous network that is resistant to flow under pressure. The process is known as gelation (Boye *et al.*, 2000). The process is of considerable importance in the food industry since it contributes significantly to the textural and rheological properties of various foods.

The mechanism of protein gelation is still not fully understood. However, the most commonly accepted process involves two steps: an unfolding process followed by an aggregation step.

1.3.1.1 Molecule unfolding

Native globular proteins such as albumen or whey proteins have a characteristic secondary structure: they contain specific amounts of α-helix, β-sheet and disordered peptide chain conformations (Kavanagh *et al.*, 2000). The reactive amino acids are located in the interior of the molecule. It is thus necessary to promote unfolding so that the reactive sites are exposed to the solvent and become chemically reactive (van Vliet *et al.*, 2004). Molecular unfolding is usually accomplished by the application of heat in an aqueous state which acts by first breaking the

hydrogen bonds, then uncoiling the polypeptide chains, thereby exposing the reactive sites (Mine, 1995). As the unfolding proceeds, the viscosity of the protein dispersion increases due to a rise in the molecular dimensions of the unfolding protein. In other instances, the heat treatment can result in the cleavage of existing disulphide bonds (Castimpoolas & Meyer, 1970) thus creating sites for new molecular bridging. With β-lactoglobulin, heat exposes the hydrophobic amino acid residues and the reactive SH group of Cys 121 to the aqueous solvent (Kerstens *et al.*, 2005).

1.3.1.2 Molecule aggregation

After the initial unfolding step, the rate and extent of aggregation determines the appearance and properties of the gel. (Dalgleish & Hunt, 1995). Various studies have shown that there are essentially two types of aggregation: (1) linear aggregation at high net charge and (2) clumped random aggregation at low net charge (Dalgleish & Hunt, 1995). More recently linear aggregation is referred to as a fine-stranded or filamentous gel and random/clumped aggregation is referred to as a coarse or particulate gel. At high net charge, aggregation proceeds slowly relative to unfolding. As such, the molecules have more time to become oriented into an ordered 3D structure (Dalgleish & Hunt, 1995). However, at low net charge, aggregation proceeds rapidly relative to unfolding because the inter-molecular repulsion is minimised. As such, a coagulum is produced and is characterised by high opacity, syneresis and low elasticity (Mulvihill & Kinsella, 1987).

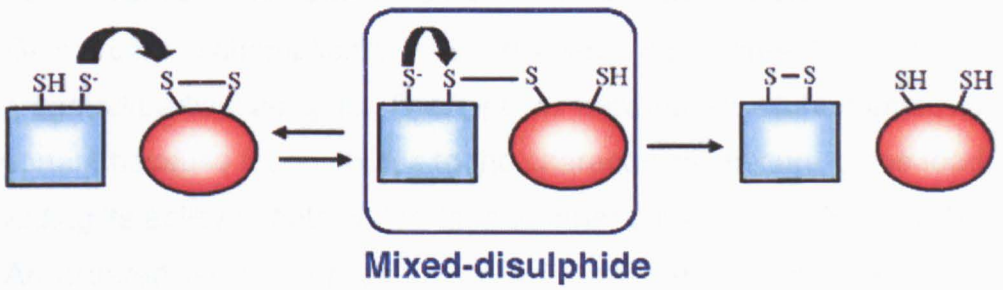
The balance of attractive and repulsive forces determines the extent of aggregation. If the attractive forces predominate, a coagulum or weak gel is formed with high syneresis. On the other hand, if weak forces prevail, no gel will be formed. Several factors affect the balance of forces and include pH, ionic strength, protein concentration, amino acid concentration, molecular weight, heating and cooling rates as well as the presence and type of other components.

One of the most common types of molecular aggregation is the formation of the disulphide bond when unfolding of the molecule occurs with the application of heat. It generally occurs via a thiol-disulphide reaction (See Section 1.3.3.2).

Although generally favoured at alkali conditions, thiol–disulphide reactions can take place at ambient and acidic conditions, given sufficient time. A thiol is a compound that contains the functional group composed of a sulphur atom and a hydrogen atom (-SH). The functional group is also referred to as the sulphydryl group. The thiol – disulphide exchange is a chemical reaction in which a thiolate group (S^-) attacks a sulphur atom of a disulfide bond SS. In fact, the sulphydryl group in its protonated form is generally un-reactive: only thiolates attack the disulphide bond. The original disulphide bond is broken, and its other sulphur atom may be released as a new thiolate, carrying away the negative charge.

Figure 1.3-A

Thiolate Induced Sulphydryl → Disulphide Reaction



It is thought that aggregates or the strands of the gel network formed at the isoelectric pH are stabilized by disulphide bonds formed slowly during the curing of a β-lactoglobulin gel (Otte *et al.*, 2000). A similar study carried out by Vasbinder *et al.* (2003) also clearly showed that thiol-disulphide bonds could be formed under acidic conditions as demonstrated in yoghurt making.

Gel formation itself is generally considered to be a two-phase process: the first phase is the formation of the primary spatial structure and the second phase is an increase in the amount of and/or stiffness

of the bonds in the gel resulting from the rapid formation of multiple hydrogen bonds (Mine, 1995; Goncalves *et al.*, 2004). After gel formation, the spatial structure does not undergo major changes as shown by a constant permeability coefficient. Increase in stiffness is shown by an increase in the elastic modulus with heating time (Verheul & Roefs, 1998). However, more recently, a proposal was made to consider gel formation as a three-stage process i.e.

1. an unfolding step, followed by
2. a step of linear fibril aggregation, and then,
3. a step of random association of the fibrils.

Evidence for the existence of linear fibrils and their interaction was provided in an electron microscope study of a homogenous soya protein gel (Clark *et al.*, 2001). Beveridge *et al.*, (1984) had suggested that the association of the fibrils in the last stage is accomplished by hydrophobic interactions, SH→SS interchange and SH oxidation within the aggregates and between aggregates and multiple hydrogen bonding.

1.3.2 Coarse/Particulate Gels And Fine-Stranded Gels.

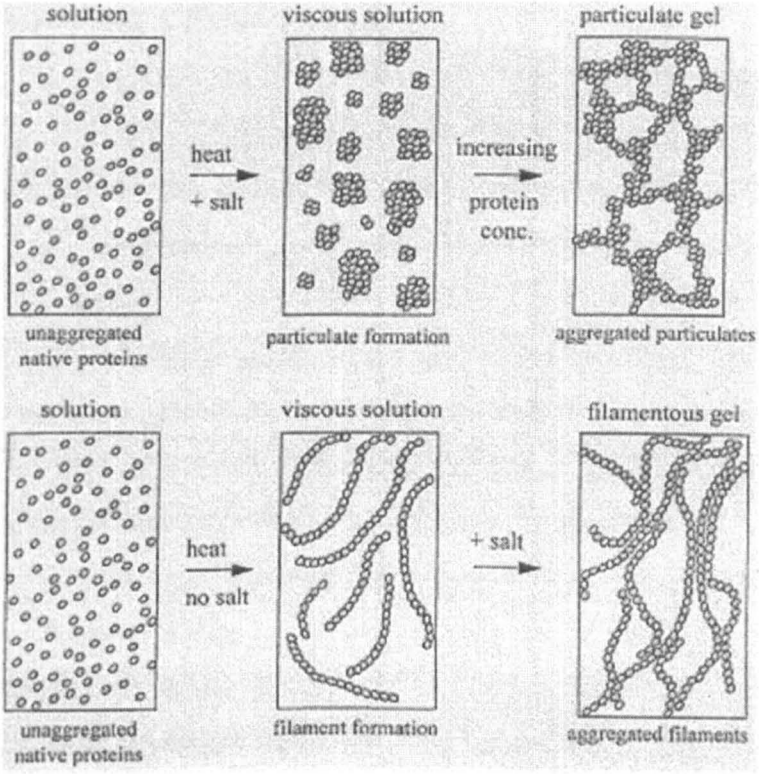
Gels can schematically be divided into fine-stranded or aggregated/particulate gels. The type of network structure formed is important because it contributes to the characteristic texture of the food including its ability to hold water, fat and other components (Fig 1.3-B).

An ordered association of molecules forms fine-stranded gels and the dimensions are often quite small so that the gels appear transparent or translucent. Conditions of strong electrostatic repulsions far from the isoelectric pH tend to favour the formation of fine-stranded gels by hindering or slowing the aggregation of the molecules. Particulate gels tend to be white and opaque and are formed in conditions of weak electrostatic repulsions close to the isoelectric point, which favours rapid aggregation of the unfolded molecules (Tavares & da Silva, 2003). Similar to other globular proteins, β-lactoglobulin can form fine-stranded gels as well as particulate gels depending on the pH and salt concentration. In the absence of added salt, particulate gels

were formed on heating at pH 4-6 whilst fine-stranded gels formed below and above this pH range (Hermansson, 1994). In a study of the rheological properties of a mixed gel of β-lactoglobulin and potato amylopectin, Olsson *et al.*, (2003) reported that the temperature at which aggregation starts has an effect on whether the resulting gel is fine stranded or particulate: the lower the temperature of aggregation, the larger the molecular clusters leading to a thicker network.

Figure 1.3-B

Schematic Diagram For Fine-Stranded and Particulate Gel Formation



Adapted from Bryant & McClements, (1998)

The mechanical properties of a gel network are defined by three factors: (1) the spatial distribution of the particles, (2) the strength of the interaction forces between the particles and (3) the structure of the particles themselves (Bremer *et al.*, 1990). The strength of the gel and the degree of cross-linking will often be altered by the inclusion of non-protein components. The gel properties will depend on the specific compound added and the type of interaction that takes place between

the protein and the compound. If the molecules in the compound aid protein-protein interaction by forming bridges or cross-links, then the gel strength will increase. If at the same time it reduces the protein-solvent interactions by reducing the net charge on the protein, then the water holding capacity may also be improved. If the extent of interaction is high or if the pH is close to the isoelectric point, its can lead to rapid aggregation which does not favour a highly cross-linked network (Artnfield, 1996).

1.3.3 Factors Affecting The Gelation Of Globular Proteins

1.3.3.1 The effect of pH

The pH has an effect on the extent of protein-protein interactions because thiol activity changes with pH (Xiong & Kinsella, 1990). In a study carried out by Mangino *et al.* (1987) it was reported that at a low pH of 4.6, the resulting gel was weak and that the structure was maintained principally by hydrophobic and ionic interactions. At this pH value, near the isoelectric point, the charge on the protein is minimal leading to reduced expansion and reduced hydration (Cheftel *et al.*, 1985). At a higher pH of 8.0, however, the thiol groups become highly reactive facilitating the formation of disulphide bridges. Even at low pH values, there is evidence that slow thiol/disulphide reactions can occur. (Shimada & Cheftel, 1989).

The behaviour of whey protein is related to the isoelectric point because at pH values below the isoelectric point of 5.2, the total charge is positive. Above this value the total charge is negative (Turgeon & Beaulieu, 2001).

In another study (Boye *et al.*, 1995) it was observed that a sample of whey protein concentrate dispersion formed a weak gel at pH values between 4 and 6. The explanation was that the absence of repulsive forces led to low expansion and low hydration. At alkaline pH values however, the whey proteins underwent extensive denaturation and expansion and the gels trapped more water. Boye *et al.* concluded that whey protein concentrate has higher thermal stability at pH 3 than at pH 9 as represented by the reduced loss of the secondary structure.

Lowering the pH of whey protein concentrate inhibits unfolding of the protein and hence increases its stability to thermal denaturation.

It was also observed in the Fourier Transform Infrared Spectroscopy (FTIR) used in this study that α-lactalbumin was denatured and formed aggregates at pH 3 and 5 but not at pH 7 or 9, whereas β-lactoglobulin both denatured and formed aggregates at pH 3 and 9. Boye *et al.* (1995) concluded that at neutral pH and higher, the aggregation and gelation of whey protein concentrates is due mainly to molecular transitions involving only β-lactoglobulin. At pH values below 7, both α-lactalbumin and β-lactoglobulin are involved in the gelation of the whey protein concentrate (Boye *et al.*, 1995).

1.3.3.2 The effect of heat

Heat has a significant effect on most globular proteins. Heat treatment can result in the cleavage of existing disulphide bonds and the activation of buried sulphydryl groups through the unfolding of the protein molecule. The activation of these groups results in the formation of new inter-molecular disulphide bonds. As such, alteration of the heat treatment conditions by modification of time and temperature will affect the gel properties by changing the rate and mechanism of denaturation and aggregation (Boye *et al.,* 1995). For any given protein, a critical concentration exists above which gelation will be observed due to improved efficiency of the molecular collisions (Bryant & McClements, 2000a).

Studies with β-lactoglobulin show that application of heat causes a free sulphydryl group in the molecule to become reactive thereby leading to a sulphydryl → disulphide exchange reaction with one of the intra-molecular disulphide bonds of an un-denatured molecule to produce a dimer. The result was the formation of an inter-molecular disulphide bond and a new free sulphydryl group is now available to propagate the reaction (Boye & Alli, 2000; Vasbinder *et al.,* 2003).

Heat induced aggregation of β-lactoglobulin was shown by Ikeda (2003) to be a two-step process at neutral pH. Granular primary aggregates are formed first, followed by subsequent aggregation of

these primary aggregates regardless of the ionic concentration. However, it was thought that the growth and the aggregation of the primary particles in the matrix were found to be concurrent processes.

1.3.3.3 The effect of ionic strength

The presence of salts strongly affects the type of gels formed especially at pH values far from the isoelectric point, where the proteins carry a large net charge. Despite differences in studies, it is clear for instance that divalent ions influence protein-protein interactions by shielding or reducing electrostatic repulsion between the proteins and also by forming protein-cation-protein bridges (Ziegler & Foegeding, 1990; Boye *et al.*, 1995). At optimum levels, these intermolecular bridges remain intact under stress whilst absorbing and dissipating the stress energy and therefore contribute to gel strength and elasticity. Above the optimum level, the formation of the inter-molecular bridges is quicker, more random and less ordered so that weaker bridging between the larger aggregates predominates. These bridges are easily broken when stressed, thus aiding syneresis, reduced gel strength and reduced elasticity (Mulvihill & Kinsella, 1988).

Boye *et al.* (1995) showed that there was a clear demonstration of the effect of NaCl on the gel characteristics of WPC. In the absence of NaCl, firm gels were obtained; below 1M NaCl, the gels were soft. No gelation was observed when the concentration of NaCl was greater than 2M. It was concluded that the presence of NaCl raised the denaturation temperature of the WPC by reducing the number of hydrogen bonds.

In another study to assess the influence of NaCl on whey protein isolate gelation, it was observed that no gel was formed when the concentration of the NaCl was below 60mM. When the concentration of NaCl in the medium was between 60mM to 150mM, the elastic modulus of the gel increased, the elastic recoverability remained high and both water loss and lightness of colour remained low. Within this range, it was proposed that the gel network consisted mostly of fine strands. When the concentration of NaCl was increased to a level

between 150mM to 200mM, elastic modulus and elastic recoverability decreased, while water loss and lightness of colour increased. In this range, it was postulated that the gel network changed from predominantly fine strands to particulates. At levels above 200mM, all four properties became insensitive to salt concentration (Chantrapornchai & McClements, 2002). The researches attributed these differences to the fact that at high salt concentrations, the strong natural electrostatic repulsion between the molecules, which would normally prevent them from coming into close proximity, is screened off. In effect, the primary impact of the NaCl is to reduce the range and length of the electrostatic interactions. Once all of the electrostatic interaction had been completely screened (at > 200mM), particle-particle interactions and gel microstructure remains constant.

When salt is added to a β-lactoglobulin solution and heated to induce gelling, the critical gel concentration was shifted to lower values. In addition a decrease in the gel temperature was observed as the salt concentration was increased (Puyol *et al.*, 2001).

Another study, carried out by Barbut (1995), suggested that $CaCl_2$ was more effective than NaCl in increasing the gel strength of whey protein isolate. Barbut further reported that the increasing presence of NaCl also had the effect of increasing the size of the protein strands, thereby reducing the water holding capacity and the gel strength.

There appears to be a clear and significant interaction between pH and the NaCl concentration. Increasing the NaCl concentration increased the gel strength and water holding capacity below pH 5, but resulted in weaker gels at pH values greater than 7. The variation in firmness is attributed to a shift in molecular structure from fine-stranded to particulate (Turgeon & Beaulieu, 2001).

1.3.4 Multi-Component Gels – Inclusion of Other Materials

In food systems, globular proteins rarely exist in isolation. They are usually surrounded by a complex mixture of different types of molecules such as salt, sugar, starches, surfactants, emulsifiers, fats, etc. Some of these compounds can form gels by themselves whereas

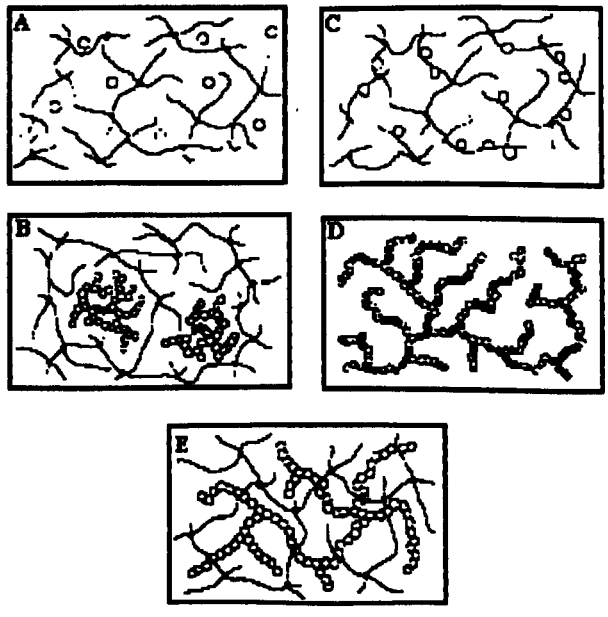
others do not. In addition they interact directly or indirectly with the proteins and can alter their functionality.

When two or more different biopolymers are subjected to conditions of molecular unfolding and aggregation, a mixed gel results (Fig1.3-C). The degree of solubility, particle size and critical concentration determines the conditions of the gel.

When the gelling agents are co-soluble and form separately continuous networks, a mixed gel is said to result. Mixed gels are often classified into one of three types: inter-penetrating, coupled and phase-separated gels. Inter-penetrating networks are formed when the two components gel separately and form completely independent networks. The networks are continuous throughout the sample but there is little or no interaction. Coupled networks are formed in the presence of favourable intermolecular interactions between the different types of polymers. Phase separated gels are formed by incompatible polymers where the molecules either repel each other or show greater affinity towards the solvent than to each other (Morris, 1986; Tolstoguzov, 1992).

Figure 1.3-C

Forms of Multi-component Gels



Key
A = phase separated gel with inactive filler particles. B = phase separated gel (multi-phase) C = phase separated gel with active filler particles. D = coupled or co-polymerized gel with heterogeneous network. E = interpenetrating gel network
Adapted from Ziegler and Foegeding, (1990)

Even when there are non-gelling materials present, they can exert an influence on the textural characteristics because the molecules or particles tend to be situated in the pores between the gel network strands where they can either serve as active or inactive fillers (Fig. 1.3-C). Active fillers are characterized by strong interactions between the filler particles and the gel matrix as they are fully incorporated into the network. The reinforcement effect becomes even more pronounced with greater amounts of the filler. By contrast, inactive fillers act as structure breakers because there is little affinity between the filler particles and the gel matrix. Increasing the volume fraction of these inactive fillers generally results in lower gel strength and causes the gel to become disrupted and weakened (Sok Line *et al.*, 2005). Sometimes, depending on the conditions within the mixture, the same filler may be active or inactive. For instance, in an experiment carried out with locust bean gum and whey protein mixed systems at a pH value close to the isoelectric point of the whey, the resultant gel was weak, suggesting that the locust bean gum was an inactive filler. However at a neutral pH, the locust bean gum had a small but positive effect on the protein network formation (Tavares & Lopes da Silva 2003). In a similar study using cassia gum at pH 7, a low concentration of the gum enhanced the aggregation rate and strength of the gel. At concentrations of the gum greater than 0.68%, the gels showed substantially lower moduli values (Gonçalves *et al.*, 2004).

1.3.4.1 Lecithin

Lecithin is mostly a mixture of glycolipids, triglycerides and phospholipids. It is used commercially in substances requiring a natural emulsifier and/or lubricant. In biochemistry, lecithin is often used as a synonym for pure phosphatidylcholine, a phospholipid that is the most abundant component of the lecithin. Lecithin is commonly extracted from egg yolk or soya protein and more recently from rapeseed.

A study carried out by Ikeda & Foegeding (1999) showed that whilst lipids generally inhibit whey protein gelation by competing with hydrophobic interaction sites, the addition of lecithin to whey protein

dispersions increased the strength of the gels. It was postulated that the increase in gel strength was accomplished by the formation of protein-lecithin complexes.

1.3.4.2 Emulsifiers

An emulsifier is a surface-active agent, which stabilizes two or more normally immiscible liquid.

The addition of surface-active agents has been shown to affect the mechanical properties and microstructure of whey protein gels. Samples of a protein gel containing surfactants such as glycerol monopalmitate (oil soluble) and polyoxyethylene sorbitan monolaurate (water soluble) showed less elasticity than gels that do not contain any surfactants. The reduced degree of flocculation of the droplets of the oil-soluble emulsifier reduces the elastic modulus of the heat-set gel. Due to the fact that the oil-soluble emulsifiers have similar reactivity as the protein molecules, they still have the ability to interact with the gel matrix. However as they will have reduced number of reactive sites, the emulsifier was only partly incorporated into the gel matrix, thereby contributing less to the mechanical properties of the resulting gel (Chen *et al.*, 2000).

In a water-soluble emulsifier, the droplets appear to completely lose their reactivity and functionality for building up a 3D network. The surface coated emulsion droplets are presented to the protein molecules as 'alien' units and therefore become excluded from the network, acting only as filler particles.

1.3.4.3 Gluten

Gluten is an amorphous mixture of protein found combined with starch in the endosperm of some cereals, notably wheat, rye and barley. It constitutes about 80% of the proteins contained in wheat and is composed of the proteins gliadin and glutenin.

The addition of gluten alters the thermal stability of whey proteins making them more heat labile and increasing the gel strength and gel elasticity. The change is accomplished by increased hydrophobic

interactions and hydrogen bonding. When the pH is at 4.2, there are also some disulphide bonds formed (Lupano, 2000).

1.3.4.4 Pectin

Pectin is a linear polysaccharide of plant origin. The chemical structure is built up from polygalacturonic acid units linked by α-(1-4) glucosidic bonds. Pectin can be partially esterified with methoxyl groups. The degree of methoxylation influences the behaviour of the pectin and is defined as the average number of methoxyl groups per percent of the galacturonic acid units. If the degree of methoxylation is greater than 50%, then it is referred to as high methoxyl pectin.

Low methoxyl pectin induced gel formation at a lower protein concentration with whey protein than was observed for whey protein alone (Beaulieu *et al.*, 2001). The same study also showed that the gel strength was affected by the concentration and degree of methoxylation of the pectin. It was postulated that a phase-separated gel was formed due to the thermodynamic incompatibility between the two systems with both the pectin and the whey proteins competing for water and calcium.

High methoxyl pectin will gel in the absence of Ca^{2+} or under acidic conditions provided the water activity is low enough (Phillips & Williams 1995).

1.3.4.5 Carrageenan

Carrageenans are a family of linear sulphated polysaccharides of alternating β-1,3- and α-1,4-linked galactose residues extracted from red seaweeds. They are large, highly flexible molecules, which curl forming helical structures. This gives them the ability to form a variety of different gels at room temperature. They are widely used in the food and other industries as thickening and stabilizing agents. There are three main commercial classes of carrageenan;

- Kappa – forms a strong, rigid gel
- Iota – forms a soft gel
- Lambda – forms a gel when mixed with proteins rather than water

At low levels (0.1%), Ќ-carrageenan is compatible with whey proteins and can be uniformly distributed into the protein network. Under such conditions the gels with added carrageenan were very similar to gel samples made from whey protein concentrate only. When calcium was added, carrageenan increased the firmness and fracturability of the protein gels. However at high levels, the mixtures changed to become more like a typical Ќ-carrageenan gel (Turgeon & Beaulieu, 2001).

1.3.4.6 Soya proteins

Legume proteins, such as soya protein belong to the globulin family of seed storage proteins called glycinin (11S) and beta-conglycinin (7S). Soya proteins are denatured in a manner similar to both egg white and whey proteins in that the hydrophobic amino acid side chains located inside the molecules are exposed to the outside by heating (Liu *et al.*, 2004; Noh *et al.*, 2005). The addition of 1 or 2% replacement of whey proteins with soya protein resulted in an increase in the storage modulus of the system. This implied that the mixed protein structure was more extensively cross-linked than the network of either protein alone. Further replacement of the whey with soya protein caused a decrease in the storage modulus of the structure due to phase separation. The maximum phase separation occurred at a 5:1 (w:w) ratio of soya/whey proteins. The incompatibility is thought to be due to the differences in molecular weight (Comfort & Howell, 2002).

1.3.4.7 Salts

Salt, in chemistry, refers to any ionic compound composed of cations and anions so that the product is neutral. When salts are dissolved in water, they are called electrolytes and are able to conduct electricity.

Salts affect the ionic nature of a gel network. They operate by screening the intra-molecular electrostatic repulsion and thereby reduce the extent of molecular unfolding (Baier & McClements, 2003). A study carried out by Arntfield (1996) demonstrated very clearly that the addition of magnesium and calcium ions to ovalbumin led to an

increase in the gel firmness. Arntfield postulated that the change was due to a modification of the attractive and repulsive forces. By choosing the optimal pH and salt concentration so as to maximize the protein-protein interaction and at the same time reduce protein-solvent interaction, it is possible to increase gel strength. However these factors need to be balanced.

Kuhn & Foegeding (1991) found that dialysed whey protein concentrate forms a stronger, more cohesive and less springy gel that was more sensitive to salt addition than non-dialysed whey protein concentrate. The conclusion was that salts can be used to alter the rheological properties of a heat induced whey protein gel.

1.3.4.8 Sugars

Scientifically, sugar refers to any monosaccharide or disaccharide. Monosaccharides are the simplest form of carbohydrates. They are the building blocks of disaccharides like sucrose and polysaccharides such as cellulose and starch. Monosaccharides contain either a ketone or aldehyde functional group and hydroxyl groups on most or all of the non-carbonyl carbon atoms. They form cyclic structures, which predominate in aqueous solution, by forming hemiacetals or hemiketals (depending on whether they are aldoses or ketoses) between an alcohol and the carbonyl group of the same sugar.

A disaccharide is a sugar composed of two monosaccharides and is formed when two monosaccharides are joined by a glycosidic bond via a dehydration reaction that leads to the loss of a molecule of water. The glycosidic bonds can be formed between any hydroxyl groups on the component monosaccharide. Specifically, a glycosidic bond is formed between the hemiacetal group of a saccharide and the hydroxyl group of some alcohol. Glycosidic bonds are fairly stable but they can be broken chemically by strong aqueous acids.

Bryant & McClements (2000) noted that the influence of sucrose on the gelation of a whey protein isolate was complex. Initially, the gelation time increased and the gelation rate decreased with sucrose concentration. However, at sucrose values greater than 6%, it was

observed that the gel time decreased and the gelation rate increased. The conclusion was that higher concentration of sucrose led to an increase in the collision efficiency i.e. the number of collisions that leads to protein aggregation.

Lactose has also been shown to slow down the denaturation of β-lactoglobulin (Renard *et al.*, 1999; Spiegel, 1999). Yamul & Lupano (2003) demonstrated that there was a positive linear relationship between the concentration of honey in the mixture and the denaturation temperature of whey protein dispersion. The Texture Profile Analysis parameters of firmness and elasticity as well as the relaxation time decreased with increasing concentrations of the honey. The conclusion was that the honey decreases the stability of the whey protein gel structure by forming hydrogen bonds with the whey protein molecules, thus decreasing the number of hydrogen bonds possible between the actual protein molecules. An increase in the number of hydrogen bonds generally leads to an increase in viscous behaviour of gels. Yamul & Lupano also postulated that honey increased the water holding capacity of the gels at all pH values by the same mechanism of forming hydrogen bonds with the water molecules.

Sorbitol has a similar effect as sucrose in stabilizing the proteins (Baier & McClements, 2003). The conclusion from this study was that due to the preponderance of the hydroxyl groups, the interaction with the protein surface was almost certainly due to extensive hydrogen bonding.

1.3.5 Milk Components

Glycomacropeptides or casein macro peptides stems from the outer layer of the casein micelle. Chymosin action during the cheese making process cleaves the glycomacropeptides from the κ-casein. The glycomacropeptides consists of a backbone of 64 amino acids. It has up to four sugar residues per molecule and a molecular weight of 7 kDA (Tolkach & Kulozik, 2005).

1.3.6 Protein Perturbing Agents

The amount of soluble protein in different solutions can provide useful information on the network structure and on the composition and types of bonds involved (Braga *et al.,* 2006). It is well established that the main contribution to biopolymer structure derive from four main kinds of molecular forces – covalent bonds, electrostatic interactions, hydrogen bonding and hydrophobic interactions (Chen & Dickinson, 1999). SDS will generally break hydrophobic bonds, urea will attack hydrogen bonds, DTE will destroy covalent disulphide bonds and dilute NaCl solution will perturb electrostatic attraction between charged molecules.

1.4 Avian Egg Albumen

Avian egg from the domesticated hen *Gallus domesticus* is arguably one of the most commonly consumed food products in the world. The global annual production was estimated 703 billion pieces in 1997 (USDA, 1997). It is also one of the most complex and complete foods found in nature. Virtually all minerals and vitamins with the exception of vitamin C are present in the albumen. The egg white, or the albumen, is the clear liquid contained within the egg. Unlike the egg yolk, it contains little fat. The biological role of the albumen in nature is to (1) serve as a physical buffer to the yolk and embryo, (2) inhibit microbial growth by the activity of lysozyme and proteinase inhibitors and (3) supply the embryo with water, protein and other nutrients. As a food material it possesses multiple functional properties such as foaming, gelling, binding and emulsification.

The egg albumen consists of about 88% water and 12% solids of which 80-90% is protein. More than 40 types of protein have been isolated with a wide range of functionality. The main ones are ovalbumin, ovotransferrin, ovomucoid, lysozyme, globulins (G2, G3), and ovomucin.

1.4.1 Ovalbumin

Ovalbumin is the major protein found in egg white (54%) and therefore, its behaviour dominates the overall performance of egg white. It belongs to the serpin class of proteinase inhibitors although unlike the majority of serpins it is unable to inhibit any proteases (Hunt & Dayhoff, 1980) Ovalbumin is classified as a phosphoglycoprotein since carbohydrate and phosphate moieties are attached to the polypeptide. Each molecule of ovalbumin contains four sulphydryl groups and one disulphide group (Li-Chan *et al.*, 1995).

Ovalbumin has a molecular weight of 45 kDa (Li-Chan *et al.*, 1995). It is a monomer and the molecule consists of a polypeptide with up to two phosphate groups per mole and a side chain of mannose and glucosamine residues. It has been reported, however, that the phosphate groups are not evenly distributed across all the molecules and that the ovalbumin can be further purified into three fractions differing in phosphorous content (Vadehra & Nath, 1973) A1, A2 and A3, which contain two, one and no phosphate groups per molecule respectively (Mine, 1995). Ovalbumin is isoelectric at pH 4.6. The denaturation temperature is 84°C as measured by differential scanning calorimetry (Donovan & Mapes, 1976).

Ovalbumin can exist in two forms: N-ovalbumin and the more stable S-ovalbumin (Huntington & Stein, 2001; Hammershoj *et al.*, 2002). The transformation from N-ovalbumin to S-ovalbumin occurs naturally and irreversibly during the storage of eggs (Vadehra & Nath, 1973). The conversion rate becomes more rapid with higher storage temperature and increasing pH (Huntington *et al.*, 1995). The S-ovalbumin is structurally more stable against denaturation than the N-ovalbumin because the molecule is more compact and has a more hydrophobic surface (Nakamura & Ishimaru, 1981). The consequence of this is that S-ovalbumin gels reach lower gel strengths compared to N-ovalbumin under conditions of constant heating (Egelandsdal, 1980). Hammershoj *et al.* (2002) confirmed that the presence of S-ovalbumin with its higher thermal stability results in a lower amount of protein unfolding and

participating in the gel network leading to a weaker gel. Fresh eggs therefore form a very soft, gritty gel with very low water holding capacity. The soft texture was reported to be due, in part, to the high carbon dioxide content, leading to the formation of carbonic acid and hence a pH closer to the isoelectric point of the albumen proteins (Hickson *et al.*, 1982). Another study concluded that for optimal gel strength, eggs should be stored for 14 days at 4°C (Hammershoj *et al.*, 2002).

Most in-shell eggs for food processing tend to be stored, with and without cooling, prior to use. The gelling properties of egg albumen from different sources could thus be different even at the same protein concentration.

1.4.2 Ovotransferrin (Conalbumin)

Ovotransferrin is the second most dominant protein (13%) in egg albumen and is also a glycoprotein. Ovotransferrin has a molecular weight of 77 kDa (Li-Chan *et al.*, 1995). It binds transition metals (Fe[III], Cu[III], Al[III]) very tightly and specifically with a binding log constant of about 15 at pH 7.0 and above. Ovotransferrin is a covalent dimer protein, composed of a N- and a C-terminal domain, each one binding one atom-ion of transition metal. Ovotransferrin is isoelectric at pH 6.1 – 6.3 and has 15 disulphide bridges but no phosphorous or sulphydryl groups (Li-Chan *et al.*, 1995). The denaturation temperature is 65°C as measured by differential scanning calorimetry (Donovan *et al.*, 1975).

1.4.3 Ovomucoid

Ovomucoid is also a glycoprotein. It is the only trypsin inhibitor found in egg albumen and constitutes about 11% of the egg white protein. It generally has a molecular weight of 28 kDa. Avian egg ovomucoid has nine disulphide bridges and no sulphydryl groups (Li-Chan *et al.*, 1995). Ovomucoid is very stable to extremes of temperature and acid. The isoelectric point is pH 4.1.

1.4.4 Lysozyme

Lysozyme represents about 3.5% of the total albumen protein. It has a molecular weight of about 14 kDa. Lysozyme is isoelectric at a highly alkaline pH of 10.7. It is antibiotic in nature and it acts by lysing the cell walls of gram–positive bacteria. It has four disulphide bridges but no sulphydryl groups (Li-Chan *et al.*, 1995) and is denatured at 75°C as measured by differential scanning calorimetry (Donovan *et al.*, 1975).

1.4.5 Ovomucin

Ovomucin is a minor glycoprotein and represents about 1–3 % of the egg albumen. It is claimed to have a randomly coiled configuration but can be fractionated into α- (carbohydrate rich) and β- (carbohydrate poor) fractions with molecular weight ranging from 135 – 720 kDa (Kato & Sato, 1971). The isoelectric point is in the region of 4.5 – 5.0. The linked carbohydrate moieties tend to form extensive hydrogen bonds with water and give rise to the characteristic gel-like structure (Li-Chan *et al.*, 1995).

1.4.6 Globulins

G2 and G3 globulins have been identified in egg white. They each comprise about 4% of the egg white proteins but have not been fully characterised. They are thought to be important in the foaming of egg albumen (Mine, 1995).

Table 1.4-1

Summary of the Characteristics of Proteins in Egg Albumen

Protein	Concentration (%)	Isoelectric pH	Molecular weight (Da)	Denaturation temperature (°C)
Ovalbumin	54	4.5	45000	84
Ovotransferrin	12	6.1	76000	61
Ovomucoid	11	4.1	28000	79
Ovomucin	3.5	4.5	8.3×10^6	Stable
Lysozyme	3.4	10.7	14300	75
G2 Globulin	4.0	5.5	4.5×10^4	92.5
G3 Globulin	4.0	4.8		66

Adapted from Li Chan et al., (1995)

1.4.7 Egg White Gelation

On heating, egg albumen undergoes a series of changes in appearance. As the temperature rises from 5°C to 45°C, the viscosity is reduced. The egg albumen begins to lose fluidity at 60°C due to the denaturation of ovotransferrin (Woodward, 1990). At 63°C, albumen starts to become turbid, a procedure accompanied by precipitation. At 66°C, the mixture becomes clear with a gelatinous mass appearing on the surface. By the time the temperature reaches 70°C, the familiar white coagulum is formed (Burley & Vadehra, 1989). Montejano *et al.* (1984) showed that there was a large increase in albumen gel elasticity between 70 – 74°C. Only a minor increase in elasticity was observed between 74 – 89°C and the elasticity actually decreased from 89°C to 91°C.The hypothesis proposed was that the development of elasticity coincided with protein denaturation and the initiation of aggregation while subsequent heating promoted the completion of the protein aggregation with increased protein cross-linking and gel strength enhancement.

Depending on the pH and salt concentration, the physical properties of the albumen gels can be significantly altered. Woodward & Cotterill (1986) reported that as the temperature and duration of heating an egg albumen sample was increased, the hardness, springiness and cohesiveness of the gel was also increased. These properties were decreased however by the addition of salt. They also noted that at pH 9, the gels had finer and more uniform microstructure than at pH 5 or 6 and that the water holding capacity was greater.

1.5 Whey Protein

Whey is the liquid remaining after milk has been curdled; it is a by-product of the manufacture of cheese or casein. Whey proteins mainly consist of α-lactalbumin, β-lactoglobulin and serum albumin. Depending on the method of manufacture, it may also contain glycomacropeptides (GMP).

1.5.1 α-Lactalbumin

α-Lactalbumin is an important component of the whey proteins and typically is about 25% of the whey protein. It has a molecular weight of about 14 kDa and the isoelectric point is between pH 4.2 – 4.5. It does not have any free thiol group that can serve as the starting point for a covalent aggregation reaction but has four disulphide bonds per monomer (Matsudomi & Oshita, 1996). As a result, pure α-lactoglobulin will not form gels upon denaturation and acidification.

1.5.2 β-Lactoglobulin

β-Lactoglobulin is a protein with a molecular weight of about 18 kDA. It is usually about 65% by weight of most whey protein preparations. It generally exists as a dimer (between pH 5.2 – 6.0) but under conditions of very low pH (< 3) and values above 6, it can exist as a monomer. As the pH of the medium is increased to between 3.4 and 5.2, the molecule becomes an octamer. The isoelectric point is 5.2. Native β-lactoglobulin has one thiol group and two disulphide bonds per monomer (Matsudomi & Oshita, 1996).

1.5.3 Bovine Serum Albumin (BSA)

BSA is a fairly large protein molecule with a molecular weight of about 66 kDa. It is about 8 –10 % of the whey protein content. The isoelectric point is about 5.8. BSA is generally very stable and although biochemically reactive, it contributes very little to the physical chemistry of whey protein.

1.5.4 Glycomacropeptides (GMP)

GMP is a highly acidic and hydrophilic peptide with a molecular weight of 7 kDa. It stems from the outer layer of the casein micelle. GMP often gets into whey due to result of the action of chymosin during cheese making, which cuts off the GMP from the κ-casein at the outer layer. Depending on the degree and type of glycosylation and genetic variants, GMP exists as a variety of sub-fractions, which may be influenced by processing conditions. Up to four sugar residues per

molecule create a partially hydrophilic character, whereas the peptide chain possesses more hydrophobic properties (Tolkach & Kulozik, 2005). Various studies have indicated that the molecular weight of GMP is pH dependent and can range up to 50 kDa at pH 7.0. One proposed explanation for the pH dependent change in molecular weight is that the GMP associates itself to form oligomers at neutral pH through non-covalent interactions and that these oligomers partially dissociate at lower pH values (Xu *et al.*, 2000).

Table 1.5-1

Summary of the Physical Characteristics of Whey Proteins

Protein	Isoelectric pH	Molecular weight (Da)	Concentration (%)	Denaturation temperature (°C)
β-Lactoglobulin	5.2	18400	60	78
α-Lactalbumin	4.8 – 5.1	14200	22	62
BSA	4.8 – 5.1	66000	5.5	64
Immunoglobulins	5.5 – 6.8	500000	9.1	72

1.5.5 Whey Protein Gelation

Under normal conditions, whey proteins form a weak but elastic irreversible gel with the application of heat. Since β-lactoglobulin is the most abundant protein in whey, it is the primary gelling agent and dominates the thermal behaviour of the total whey system. On raising the temperature, the dimer structure dissociates into monomers (McKenzie & Sawyer, 1967). Above 60°C, the monomer is partially unfolded, thereby exposing the non-polar groups and the buried thiol group (Hoffmann & van Mil, 1999). Recent studies suggest that the unfolding of the proteins by heat begins at 40°C and proceeds slowly such that at 62°C, only 10% denaturation has occurred. Above 65°C, the degree of denaturation follows a linear relationship with temperature and is 95% complete at 85°C (Parris & Baginski, 1991).

Once unfolded, aggregation then depends on the protein concentration and the pH. At alkaline pH, the aggregation step of gel formation is mainly attributed to β-lactoglobulin. The functional

properties of whey proteins depend on their composition as well as the degree of denaturation. Nevertheless there appears to be a synergistic effect between all the protein fractions of whey protein. For instance in a study carried out by Matsudomi *et al.* (1991), it was demonstrated that while an 8% α-lactalbumin solution did not gel, a combination of 2% β-lactoglobulin and 6% α-lactalbumin resulted in gelation, even though each protein individually could not form a gel at these concentrations. Proteins are denatured to some extent by the various processes used in their isolation and production and as a consequence, two protein ingredients with the same composition can exhibit very different functional properties due to the differences in the degree of denaturation (Bryant & McClements, 1998).

The functionality of one component may be significantly altered by the presence of other macromolecules in the system. For instance, bovine serum albumen exhibits linear aggregation at pH 7 and low NaCl concentration. It possesses excellent gelling characteristics (Murata *et al.*, 1993). β-Lactoglobulin gel formed at pH>6 with no added salt forms small aggregates and is transparent (Stading *et al.*, 1992).

According to the literature, in addition to the degree of unfolding, the structure and properties of whey protein gels will also vary depending on the type and kinetics of the aggregation process and the nature of the bonds – covalent or non-covalent. The covalent bonds are inter-molecular disulphide bonds, which are formed in disulphide and sulphydryl exchange reactions. The non-covalent bonds are due to van der Waals forces, electrostatic interactions, hydrogen bonds and hydrophobic interactions (Roefs & de Kruif, 1994).

Another important variable is the pH of the gelling medium. Boye *et al.* (1995) reported that whey protein was more extensively denatured by heating at the alkaline side of the isoelectric pH but more stable when heated at pH values on the acidic side of the isoelectric region. Fourier transform infrared spectroscopy also showed clearly that the whey protein loses more of its secondary structure and unfolds more extensively at pH 9 than at pH 3.

1.5.5.1 Cold setting

Several studies have been reported in which cold gelation is induced with Ca^{2+}. First, a solution of native whey proteins is heated to a temperature where the molecules partially unfold. The properties of the solution are carefully controlled so that the proteins aggregate into filaments or strands but do not form a three-dimensional structure. The conditions required are low protein concentration and an absence or very low levels of salt (Hongsprabhas & Barbut, 1997a). Once the solution has been prepared in this manner, it is cooled down and then used directly or dried into powder form and used later. After this unit operation, salt was added to induce gelation at temperatures below 24°C. It is also known that calcium levels up to about 10mM increase the strength of whey protein gels due to the electrostatic interactions with the negatively charged and now unfolded protein molecules (Mulvihill & Kinsella, 1988). The gel formed by this method has a fine-stranded structure, better water holding capacity and higher gel strength than one formed by the conventional heat-induced method at the same salt level.

Van Camp *et al.* (1997) reported that whey protein gels formed at neutral and alkaline pH are stabilised by disulphide bonds. Hongsprabhas & Barbut (1996) reported that the polymerisation of whey proteins can occur via sulphydryl-disulphide interchange reactions and that the extent of the polymerisation is time-dependent. They also concluded that protein suspensions heated to 90°C as opposed to 70°C had more open conformation and interactions amongst the protein molecules, giving rise to a clearer gel. Clearer gels are usually formed when aggregation or polymerisation is slow (Taylor *et al.*, 1994). Alting *et al.* (2003) observed that it is the number of thiol groups rather than the size of the aggregates that determines the hardness of cold set whey protein gels.

1.6 Synergies Between Egg Albumen And Whey Protein Gels

In a study reported by Ngarize *et al.* (2004), there was clear evidence of synergy between the molecules of whey protein and albumen in a mixture. For high deformation tests, the highest Young's Modulus was obtained for a sample of 10:5 whey/egg albumen mixture as compared to a 5:10 or 7.5:7.5 mixture. In addition, a calculated 'interaction index' was highest for this mixture at 179% as opposed to 56% and 16% for the other 2 samples respectively.

The conclusion was that the synergistic effect depended on the degree of unfolding of the individual proteins in the mixture.

1.6.1 Water Holding Capacity

The permeability and hence the water holding capacity of a gel depends on the shape, number and size of pores in the network. If the spatial structure of the gel is changed, then it can become easier or more difficult for water to flow through the structure (Verheul & Roefs, 1998). In general, the more open the structure, the lower the water holding capacity. Particulate gels tend to have a lower water holding capacity than fine-stranded gels (Hermansson, 1994).

How well the water is held in the gel network can be determined by the manner in which the water exudes from the gel when subjected to physical compression. The effect of the physical force is to create minute cracks and flaws in the gel network. As more energy is supplied to the growing crack propagation, the propensity to fracture increases with the concomitant exudation of the water stored in the pores.

The added ingredients can affect the gel in two ways. They may either (1) physically affect the microstructure of the gels by changing the way the gel is formed due to alteration of the unfolding and aggregation mechanics or (2) they may be in competition with the protein for the available water.

1.7 Food Texture

There is no doubt that texture plays an important part in human appreciation of food. The textural characteristics of foods, resulting from a combination of physical and chemical properties, constitute one of the two main sensory attributes perceived by consumers (Turgeon & Beaulieu, 2001), the other being flavour. The diversity of food structure is based on the way in which specific interactions take place between the various biopolymers that make up the food system. Controlling these polymer interactions is of key significance in the development of novel foods and processes. Texture, in itself, is a group of physical properties that derive from the structure of the food material and the way in which its constituent ingredients interact. An understanding of the textural properties of a food material can be achieved by examination of its rheological behaviour and microstructure, as long as consumers can relate them to the perception of texture (Bourne, 2002). Correlations of instrumental analysis and sensory data are limited to individual products and often cannot be extrapolated to other products. Nevertheless, textural properties obtained from Texture Profile Analysis (TPA) measurements have been well correlated with the sensory evaluation of textural parameters (Lau *et al.*, 2000) and are useful in routine analysis of gel texture. The perception of texture constitutes how the food quality is judged and is an important factor in whether the food is accepted or rejected. All the senses interact to make this judgement – sound, touch, smell, appearance.

Scott-Blair (1958) categorized the instrumental techniques used to measure food texture into three groups – empirical, imitative and fundamental.

1.7.1 Instrumental Techniques For Measuring Food Texture

1.7.1.1 Empirical tests

Empirical tests measure something physical under well-defined conditions. By their very definition, empirical tests are developed by

experimentation and observation and thus often lack a rigorous scientific basis (Rosenthal, 1999). However, this does not disqualify their use and in some sectors of the food industry empirical tests act as standards for assessing food quality.

1.7.1.2 Imitative tests

Imitative tests attempt to simulate the conditions to which the material is subjected in the mouth. One of such is the Texture Profile Analysis (TPA) created at General Foods in the 1960s. In a series of papers, Szczesniak (1963), defined a range of textural terms and established sensory standards that enabled anyone to consider each of the textural attributes at distinct, defined levels.

1.7.1.3 Fundamental tests

Fundamental tests measure well-defined, innate physical properties such as viscosity or elastic modulus. They do not depend on the shape of the test sample, the conditions of loading or the apparatus. Such tests are scientifically rigorous and the data is expressed in scientific units.

Nevertheless, food texture measurement is essentially a human experience. Whilst data can be collected from subjecting a food material to a physical test, results of such may not necessarily correlate with human perception of texture.

1.7.2 Application Of Engineering Principles To Food Texture

Instrumental methods for measuring texture are becoming increasingly important. Many of the mechanical tests commonly employed nowadays based on compression, tension, shear, stress relaxation and fracture mechanics were originally developed by engineers for application with materials utilised in construction such as steel and concrete (Szczesniak, 1963; Vincent, 2004). Such engineering materials are brittle and elastic. Food materials behave quite differently. Most foods, being biological in nature, do not act like engineering materials and will flow and change shape dramatically

under load: many times permanently (Rosenthal, 1999). In spite of these differences, Mohsenin (1970) advocated objective fundamental engineering definitions of the mechanical attributes associated with the texture of food materials. In engineering materials, the strength of the materials is often what is of concern. In foods however, most of the tests have to do with the structural weakness of the food material.

The sensory assessment of food texture depends on the specific properties of the food: its arrangement into cells, fibres and pores and the interaction thereof during mastication. When eaten, food morsels are deformed in the mouth by biting and chewing. As a result of this deformation, the food material will respond differently to the stresses induced depending on its shape and size, its inherent properties, the rates of biting, chewing and orientation. In a mechanical test, a small piece of the material is deformed in a controlled manner and both the applied force as well as the displacement of the object is measured. A force-displacement curve can thus be obtained. Sample size can be eliminated as a variable by calculating the stress (force/area) and strain (change in dimension/original dimension). The concepts of stress and strain were developed for engineering materials that are normally brittle, stiff and elastic: large stresses result in very little strain.

1.7.3 The Concept Of Deformation, Stress And Strain

When a force is applied to solid matter, stress will develop in the material according to the nature of the force and the area over which it is applied. True stress is defined as the force per undeformed cross sectional area

$$\sigma = \frac{F}{A_o}$$

F is the applied force and A_o is the original, undeformed cross-sectional area.

1.7.3.1 Stress

Stress is the outward expression of the restoring force developed by stretching the inter-atomic bonds holding the material together.

For engineering materials where the modulus of elasticity is high, this is certainly true. However for inelastic or viscoelastic materials and when the deformation is large, the true stress can differ significantly from this nominal or engineering stress. (Tang *et al.*, 1996,) The true stress or Hencky stress (ε) is a ratio of the force and the cross sectional area of the deformed material and for such incompressible materials, a correction has to be made for the change in the area.

1.7.3.2 Strain

Strain is a dimensionless number and is a ratio of the shape before and after deformation

$$\text{Strain} = \frac{\Delta L}{L}$$

ΔL is the deformation and L is the original length

As with stress, small strain values are adequate. But in extensible materials where they can be deformation to very large strain values, a correction factor is often applied to compensate for the changes in dimension during stretching. The correction is referred to as the Hencky strain (ε_H)

$$\varepsilon_H = \ln (L/Lo)$$

L is the length after the stress has been applied and Lo is the length of the unstressed material.

1.7.3.3 Deformation

In a perfectly elastic solid, there is an immediate and finite deformation when a force is applied to the material. The change is instantaneous and as the force is increased, the amount of deformation increases directly in relation to the applied force. When the force is removed, the material recovers its original shape and size. In a perfectly viscous material, usually a liquid, the material begins to flow as soon as the deforming force is applied. The rate of flow is proportional to magnitude of the force applied. The material continues to flow as long as the force is applied. There is no recovery of shape when the deforming force is removed. In a perfect plastic material,

deformation does not begin until a certain value of stress is attained. Deformation is then permanent and there is no recovery of shape when the force is removed.

In a visco-elastic solid, there is an instantaneous deformation when the deforming force is applied and then the material continues to deform as long as the force is present. When the force is removed, there is some recovery of the original shape (due to the elastic component) but not a full recovery (due to the viscous component).

Most foods exhibit both elastic and plastic or flow behaviour simultaneously and are described as visco-elastic. A visco-elastic material has the following properties

1. Hysteresis: as seen in the stress-strain curve.
2. Stress relaxation occurs: constant strain decreases the stress
3. Creep occurs: constant stress increases the strain

Visco-elasticity differs from plastic behaviour/flow and therefore, their properties will depend on the rate of strain and how quickly the instrument measures the corresponding stress. When a force is applied to a visco-elastic food material, there is an immediate compression of the food sample, which is the instantaneous elastic deformation. The deformation is followed by a continuous but decelerating rate of deformation called 'creep'. When the force is removed, there is some recovery but the product does not recover its original shape and size (Bourne, 2002).

Plastic deformation and flow occur in certain materials that exhibit elastic deformation at low stresses until the material is stressed beyond its yield point, after which it flows like a liquid. At applied stresses greater than the yield stress, the internal structure may be disrupted thereby allowing fluid motion.

Stress-strain relationships in the elastic region before failure can reveal the interactions among the functional components of the materials. The simplest model for elastic materials in small deformation is Hooke's law, which states that for materials under low to moderate strains, the stress and strain values are proportional and that when the stress is removed, the material instantly returns to its original form.

Methods for obtaining stress-strain relationships in large deformation include uniaxial tension, uniaxial compression and torsion tests (Tang *et al.*, 1997). Uniaxial compression tests are widely used for their simplicity. Tension tests are more difficult to perform because of the difficulty in mounting samples. Torsion tests are becoming increasingly popular (Diehl *et al.*, 1979; Vigdorth & Ball, 1998). The experimental results from each of these tests often reflect different aspects of the behaviour of the food material and as such it may be possible to obtain more comprehensive information of the food by using all three methods. These results are quite obvious in an experiment carried out by Mine (1996). There were clear differences reported in the rheological properties of gels made from succinylated ovalbumin as compared to ovalbumin. The succinylated ovalbumin gel showed lower residual strain i.e. they were more elastic, than the ovalbumin gel.

1.7.4 Compression Tests

Compression tests include texture profile analysis, stress relaxation and puncture tests.

It is intended that in the present study, instrumental test methods will be used to characterise the differences between the albumen and whey protein gels. While various tests have shown that differing treatments produce diverse gel characteristics, what is not yet known is how well these tests correlate with human sensory perception/acceptability when applied to meat-free products. It will be foolhardy, in this context, to perform a series of tests that show significant differences in specific attributes but are imperceptible or unimportant in a human subject. Equally it is important to mention that a high correlation between attributes by itself does not necessarily confirm a cause and effect relationship.

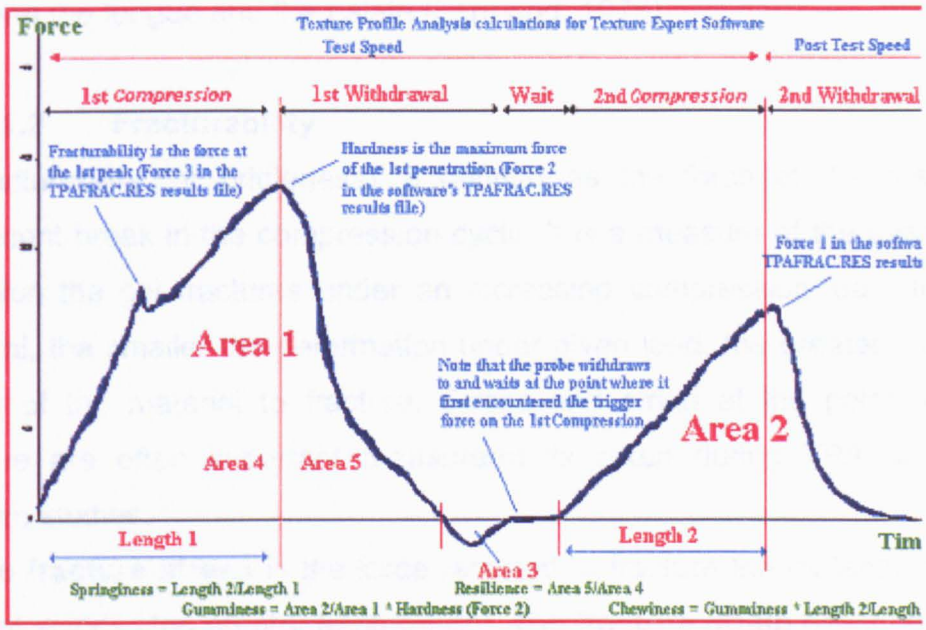
1.7.4.1 Texture Profile Analysis

Texture Profile Analysis (TPA) is an objective method of analysis pioneered by Szczeniak (1963) and later refined by Bourne (1978) in which a mechanical device is used to compress a bite-sized piece of food. Following compression, the force-time curve resulting from this

simulation is analysed. TPA is based on the recognition of texture as a multi-parameter attribute. The test consists of compressing a bite-size piece of food two times in a reciprocating manner that imitates the action of the human jaw. From the curve, a number of textural parameters are derived that correlate well with sensory evaluation.

Figure 1.7-A

Typical Texture Profile Analysis Graph



Adapted from www.texturetechnologies.com/.../tpa2.gif

In addition to these sensory standards, a compressive-force deformation instrument was described. A flat end plunger was used to compress the food sample. A two-bite cycle was used and the stress that developed in the food sample was measured as the sample was being compressed. After the first bite, the load was removed and the sample allowed to relax slightly. The second bite compressed the food sample again before allowing it to relax a second time. The resistance to deformation was monitored throughout this two-bite cycle. From the stress-strain TPA curve various parameters were calculated as shown in Fig. 1.7-A.

1.7.4.1.1 Hardness

Hardness is defined as the force necessary to attain a given deformation and is a measure of the maximum peak attained during the first compression of the compression cycle. It is related to the strength of the gel structure under compression. The hardness does not necessarily occur at the point of deepest compression although it does for most products (Bourne, 1982). In sensory terms, it refers to the force required to compress a substance between molar teeth or between the tongue and the palate (Larmond, 1976).

1.7.4.1.2 Fracturability

Fracturability (or brittleness) is defined as the force at the first significant break in the compression cycle. It is a measure of the ease at which the gel fractures under an increasing compression load. In general, the smaller the deformation under given load, the greater the ability of the material to fracture. Stress and strain at the point of fracture are often important measurements taken during TPA and fracture studies.

The **fracture stress** is the force required to fracture the material: a low value indicates greater fracturability. The **fracture strain** describes the deformability and may also give an indication of the cohesion properties: a higher value indicates greater deformability. In sensory terms fracture is a product of a high degree of hardness and a low degree of cohesiveness (Larmond, 1976).

1.7.4.1.3 Springiness

Springiness is related to the height the sample recovers during the time that elapses between the end of the first bite (compression) and the start of the second bite (compression). It is typically measured as the distance of the detected height of the product on the second compression (Length 2), divided by the original compression distance (Length 1). In reality, it is a **measure of the elasticity** of the product and is the rate and extent to which deformed material goes back to its undeformed state when the deforming force is removed. High

springiness will result when a gel structure is broken into a few large pieces during the first TPA compression, whereas low springiness results from the gel breaking into many small pieces. From a sensory point of view, it measures the degree to which a product returns to its original shape once it has been compressed between the teeth (Larmond, 1976).

The importance of the springiness is also related to the way in which water is held within the gel structure. If a gel loses a lot of water during compression, then it will be difficult to return to its original shape.

1.7.4.1.4 Chewiness

Chewiness can be defined as the energy required in masticating a solid food to a state in which it is ready for swallowing. As mastication involves chewing, cutting, shearing, piercing, grinding along with saliva lubrication, it is a difficult parameter to measure directly. Chewiness is a product of hardness, cohesiveness and springiness.

1.7.4.1.5 Cohesiveness

Cohesiveness is a measure of the rate at which a product disintegrates under mechanical action or the degree of difficulty in breaking down the gel's internal structure. Cohesive products keep their integrity in the mouth for longer periods (Adhikari *et.al.*, 2002). It is calculated as the ratio of the positive force area of the second compression (Area 2) to the first compression (Area 1). In sensory terms, cohesiveness refers to the degree to which a substance is compressed between the teeth before it breaks (Larmond, 1976).

1.7.4.1.6 Resilience

The resilience is a measure of how the sample recovers from the compression both in terms of speed and forces derived and has been referred to as a form of 'instant springiness'. It is a ratio of the area from the first probe reversal point (Area 5) to the crossing of the x-axis and the area produced from the first compression of the cycle (Area 4).

1.7.4.1.7 Gumminess

Gumminess is very similar to chewiness but is defined differently as the energy required to disintegrate a semi-solid food to a state ready fro swallowing. It is a product of a low degree of hardness and a high degree of cohesiveness and is calculated as such from the hardness and cohesiveness terms. In sensory terms, it refers to the denseness that persists throughout mastication (Larmond, 1976). Gumminess is mutually exclusive with chewiness since a product would not be both a semi-solid and a solid at the same time.

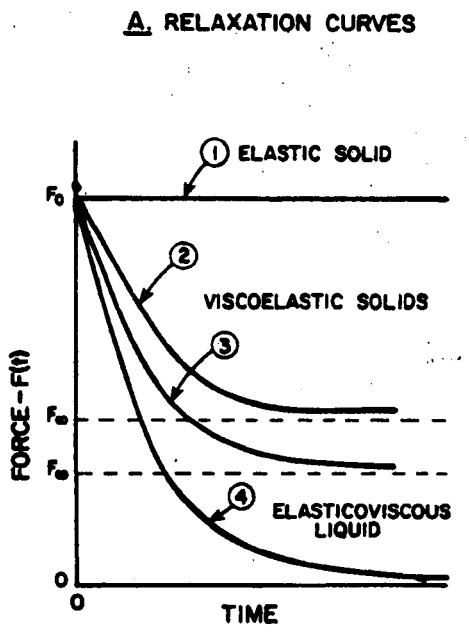
While the development of TPA has provided a valuable aid to assessing food texture, researchers state that care should be exercised in accepting the results for purposes other than comparative evaluation (Rosenthal, 1999). The reason is that although the technique imitates what goes on in the mouth, there are still some fundamental differences between what happens in a test rig and what actually happens during mastication. Examples include temperature control and lubrication by salivation. In addition, the relationship between some of the sensory characterises and the TPA parameters are not necessarily linear.

1.7.4.2 Stress Relaxation

Stress relaxation is defined as the decrease of stress with time in a material placed under finite and constant strain. It is calculated by subjecting a material to a specific level of deformation. The change in force is measured until a state of equilibrium is achieved. In many instances, the time taken to reach the asymptote value is excessive, in which case a lower value is taken as the relaxation time. In a perfectly elastic solid, all of the energy is stored and there is no stress decay due to the fact that the material will regain its original shape when the force is removed. On the other hand, in a perfectly viscous material, the stress eventually decays to zero, as the material is not able to recover its shape when the compressing force is removed. Most food materials demonstrate both viscous and elastic behaviour and are said

to be viscoelastic. In viscoelastic materials, the applied stress will level off to a value greater than zero but less than the force applied (Fig 1.7-B).

Figure 1.7-B
Typical Stress Relaxation Plot



Adapted from Peleg, 1979

In a series of calculations conducted by Peleg & Moreyra (1979) two parameters were derived from the normalization stress relaxation curve. These parameters ***a*** (relaxation level/residual stress) and ***b*** (relaxation rate) were regarded as empirical shape characteristics of the experimental curve. While they of themselves are devoid of physical significance as absolute values, the numerical value of the constant *a* was defined as a measure of what portion of the initial stress is relaxed. It thus gives an indication of the general mechanical character of the material. When the ***a*** constant has a value near to zero, it corresponds to a more elastic solid. Higher values are indicative of viscous behaviour (Buffa *et al.*, 2001). The constant ***b*** represents the steepness of the decay (1/*b* is the time to reach the level of *a*/2).

Foods are materials and therefore have the mechanical properties of materials (Dobraszczyk & Vincent, 1999). The overall texture is

governed by a combination of mechanical and fracture properties. In material science, the objective is to apply some type of mechanical deformation to the food sample, measure the responses and then try to correlate the response with the results of a sensory panel. At the heart of the materials approach lies the idea that the mechanical properties of a food and its internal structure are correlated. As such the mastication conditions in the mouth are directly related to the perceived texture of that food during eating. Specifically, viscoelasticity is a molecular rearrangement. When stress is applied to a visco-elastic material, parts of the molecular chain change position. This movement or rearrangement is called creep.

1.7.4.3 Creep

Creep is the term used to describe the tendency of a material to strain, move or to deform permanently to relieve constant stress. Like stress relaxation tests, creep tests can provide information about texture in visco-elastic materials. In creep tests, the sample is subjected to compression up to a constant stress. As shown in Fig 1.7-C, the creep curve can be subdivided into three distinct regions: OA, AB and BC. OA represents the region where elastic deformation occurs. If the stress is removed anywhere within this region, the sample will recover its original structure. The AB region represents the time-dependent retarded elasticity. During this stage, filaments and bonds in the structure begin to rupture as the rate of strain gradually increases. The proportion of breaking bonds increases until point B is reached. The BC region is a linear region associated with viscosity deformation in which some of the bonds are permanently ruptured. If the stress is removed after point C, there is a degree of recovery, depending on the extent of structural damage (Xu *et al.*, 2008).

1.7.5 Tensile Tests

Tensile tests are not commonly used in foods, because when consumed by mastication in the mouth, compression, not tension is the most common force. They tend to be most commonly used when the

stickiness or adhesiveness needs to be assessed. Nevertheless, a few are in use as subsequently described.

Guinee & O'Callaghan (1997) performed tensile tests on Mozzarella cheese by melting the cheese on a horizontal platform. After the ends of the cheese were locked in position, a motorized winch drew the roller mounted half of the platform along the rail until the cheese sheet broke. The distance to which the cheese was stretched before it broke was measured.

Reyes-Vega *et al.* (1998) measured the tearing strength of corn tortillas by cutting strips, angling them at 45° from the vertical and pulling at a constant speed until rupture occurred.

Olsson *et al.* (2000) described a method for tensile fracture test in which samples of β-lactoglobulin gels were glued to plates covered with sandpaper and clamped to an Instron machine. The pieces were then stretched until fracture occurred.

McEvoy *et al.* (1985) carried out a different type of test using ring-shaped gelatin gel specimens. The tensile test was accomplished by hanging the ring sample over two dowel pins: one mounted on the transducer stage and the other on the driven stage of the test machine. Stress was calculated on the basis that the tension in each leg was half the measured force. Strain values were derived from the increase in ring circumference equalling twice the increase in pin separation.

Scanlon & Long (1995) carried out a tensile test of I-shaped cuts of potato. The ends were attached to two parallel plates using high resin glue. Test pieces were than stretched until fracture occurred.

1.7.6 Torsion Tests

Torsion tests are those in which a force is applied that tends to rotate or twist the object around an axis with respect to the other parts. Tang *et al.* (1997) describe a torsion test performed on gellan gels in which both ends of the gel were fastened with cyanoacrylate glue and twisted at a constant strain rate in a Bohlin rheometer. The shear stress and the corresponding shear strain were then calculated. Studman & Yuwana (1992) developed a torsion test for measuring the

firmness of fruits by impaling the fruit on a spindle, rotating it against a rod and thus measuring the crushing strength of the flesh.

Farouk *et al.* (2002) described a torsion test carried out on cooked meat batter gels. The samples were machined into a dumb-bell geometry and torsionally sheared to the point of failure in a device attached to a Brookfield viscometer.

Diehl *et al.* (1979) designed a torsion test attachment for measuring structural failure in apple, potato and honeydew melon. The methods they developed allowed for the measurement of both engineering and Hencky stress and strain at failure. Ikeda & Foegeding (1999) performed the same test on whey protein isolate gels.

Both Hamann *et al.* (1983) and Montejano *et al.* (1984) severally compared torsion and compression tests for egg albumen and modified egg albumen gels.

The advantage of a torsion test is that it produces a pure shear stress and thus maintains shape and volume during the test. It also allows for the creation of tension, compression and shear in equal magnitudes (Bourne, 2002).

1.8 Other Engineering Concepts As Applied To Food Materials

1.8.1 Stiffness

Stiffness is a measure of how difficult it is to deform a material and is expressed as the ratio between stress and strain (Young's Modulus, E). Although the Young's modulus of elasticity is often used to describe foods, there is a school of thought that this term should be preserved and only used in accordance with accepted rheological definitions. Since very few foods are perfectly elastic, it has been postulated that the term modulus of deformity (or Apparent Young' s modulus) should be used for viscoelastic materials (Mohsenin & Mittal, 1977; Bourne, 2002).

$$E = \frac{\sigma}{\varepsilon}$$

For a spherical body under axial loading, the value of E can be calculated as

$$E = \frac{0.531F(1-\mu^2)}{D^{1.5}} \left[\frac{4}{d}\right]^{0.5}$$

F is the force corresponding to deformation D, d is the diameter and μ is Poisson's ratio (Mohsenin, 1978).

Most materials demonstrate linear Young's Modulus at small strain values at which point they are behaving in an elastic manner and will return to its original dimensions once the stress is removed. Beyond a certain stress value, the limit of elasticity, the stress strain relationship becomes disproportionate and in some instances, non-linear.

1.8.2 Strength

The strength is the maximum stress at which the material breaks. It is often referred to as the yield point and is characterised by a yield strain and yield stress value. In some products, the fracture does not occur at the yield point. In such cases, the yield point refers to a significant change in the slope of the stress-strain curve.

1.8.3 Hardness

Hardness is the maximum force obtained at a given deformation. The material may or may not have fractured by the time the given deformation is attained.

1.8.4 Toughness

Toughness is defined as the resistance to cracking i.e. the energy required to propagate a fracture by a given crack area (Dobraszczyk & Vincent, 1999). As such a strong material is not necessarily tough. Glass and ceramics are strong: they require a large force to break but the deformation is small. Wood is tough but not strong: it breaks at a much lower force but at much greater deformation. Muscle is much weaker and breaks at a very low force but has high toughness as demonstrated by ability to stretch and deform. In a stress-strain curve,

toughness can be calculated as the total area under the stress-strain curve. This area is an indication of the amount of work per unit volume that can be done on the material without causing it to fracture.

1.8.5 Poisson's Ratio

Poisson's ratio is the ratio of lateral strain to axial strain and is defined by the following equation

$$\mu = \frac{\Delta D/D}{\Delta L/L}$$

where D is the width of the specimen and L is the length. ΔD and ΔL are the changes caused by the application of the stress. (Bourne, 2002).

If the volume is unchanged, the Poisson's ratio is 0.5. If less than 0.5, the volume changes. Since water is virtually incompressible, foods with high water content have a Poisson's ratio close to 0.5.

1.8.6 Fracture Mechanics

The study of the fracture mechanics of foods is a useful branch of materials science that can yield information of more general interest. It can be a useful way of distinguishing between such concepts as hardness/crunchiness and crispness (Vincent, 2004). Fracture mechanics, as a field of study, is also quite useful to food structuring agents as it can be related to the sensorial perception of the consumers and is measurable by physical and mechanical properties (Turgeon & Beaulieu, 2001). Fracture is considered to occur when the bonds between the structural elements of a material break, resulting in catastrophic failure of the material (Rosenthal, 1999). Griffith (1921) stated that all materials contain minute cracks and flaws and that some materials are more sensitive to the presence of these cracks than others. In brittle materials, stress is concentrated locally around the crack tip, building up very high stresses and an excess of energy that allows the crack to propagate. In plastic materials, the stress around the crack is limited by flow, leading to intense deformation around the crack tip, resulting in blunting and preventing the crack from growing. For a crack to grow, energy must be supplied to it from the surrounding

stress material, where the energy is stored as strain. Fracture occurs when the rate at which the strain energy is released per unit crack exceeds the rate at which surface energy is absorbed in creating new fracture surfaces. Thus the critical energy for fracture is dependent on the stress, the mode of cracking, the elastic modulus and the sample geometry (Lowe *et al.*, 2003).

1.9 Microscopy

Confocal Laser Scanning Microscopy (CLSM) is a relatively new optical tool that is being increasingly used in the food analysis (Durrenberger *et al.*, 2001). Confocal microscopy offers several advantages over conventional optical microscopy. Some of these advantages include controllable depth of field, the elimination of image-degrading out-of-focus information and the ability to collect serial optical sections from thick specimens without the need to slice (Mellema *et al.*, 2000). The CLSM detects in-focus regions only; the out of focus parts appear black and this is why the application of CLSM is not limited to thin samples. The key to the confocal approach is the use of spatial filtering to eliminate out-of-focus light or flare in specimens that are thicker than the plane of focus. By having a confocal pinhole, the microscope is really efficient at rejecting out of focus fluorescent light. In CLSM, as opposed to conventional microscopy, the sample is moved relative to the microscope in order to obtain an image with enhanced resolution. The practical effect of this is that the image comes from a thin section of the sample. The resulting stack of images is then subjected to a deconvolution process, which is a newly developed procedure to de-blur the images. CLSM thus enables samples to be observed using minimal preparation procedures because of its unique optical sectioning capabilities and high spatial resolution. By scanning many thin sections through the sample, it is possible to build up a very clear three-dimensional image of the sample.

One of the other advantages of CLSM is that visualization of a sample can occur at ambient conditions and thus allows observation of the sample in its hydrated state. A further advantage is the possibility to follow, in situ, the dynamics of processes such as phase separation, coalescence, aggregation, coagulation, etc. (Durrenberger *et al.*, 2001).

In a conventional wide field microscope, the entire specimen is bathed in light from a mercury or xenon source, and the image can be viewed directly by eye or projected onto an image capture device or photographic film. By contrast, the method of image formation in a confocal microscope is fundamentally different. Illumination is achieved by scanning one or more focused beams of light, usually from a laser or arc-discharge source, across the specimen. This point of illumination is brought to focus in the specimen by the objective lens, and laterally scanned using some form of scanning device under computer control. The sequences of points of light from the specimen are detected by a photo multiplier tube (PMT) through a pinhole (or in some cases, a slit), and the output from the PMT is built into an image and displayed by the computer.

1.9.1 Labelling

Although unstained specimens can be viewed using light reflected back from the specimen, they usually are labelled with one or more fluorescent probes. Biological materials rely heavily on fluorescence as an imaging mode. Many fluorescent probes are constructed around synthetic aromatic organic chemicals designed to bind with a biological macromolecule such as protein. The fluorescent dyes are labelling agents that contain excitable structures that emit fluorescence after illumination by light of a specific wavelength. As such, the fluorescent dyes are useful in monitoring cellular integrity (live versus dead and apoptosis), endocytosis, exocytosis, membrane fluidity, protein trafficking, signal transduction and enzymatic activity. In addition, fluorescent probes have been widely applied to genetic mapping and chromosome analysis in the field of molecular genetics.

In certain instances, the naturally occurring fluorescence may be sufficient to generate a contrast. Foster *et al.* (1997) reported such a situation with the use gelatin as an emulsifier in a biopolymer emulsion.

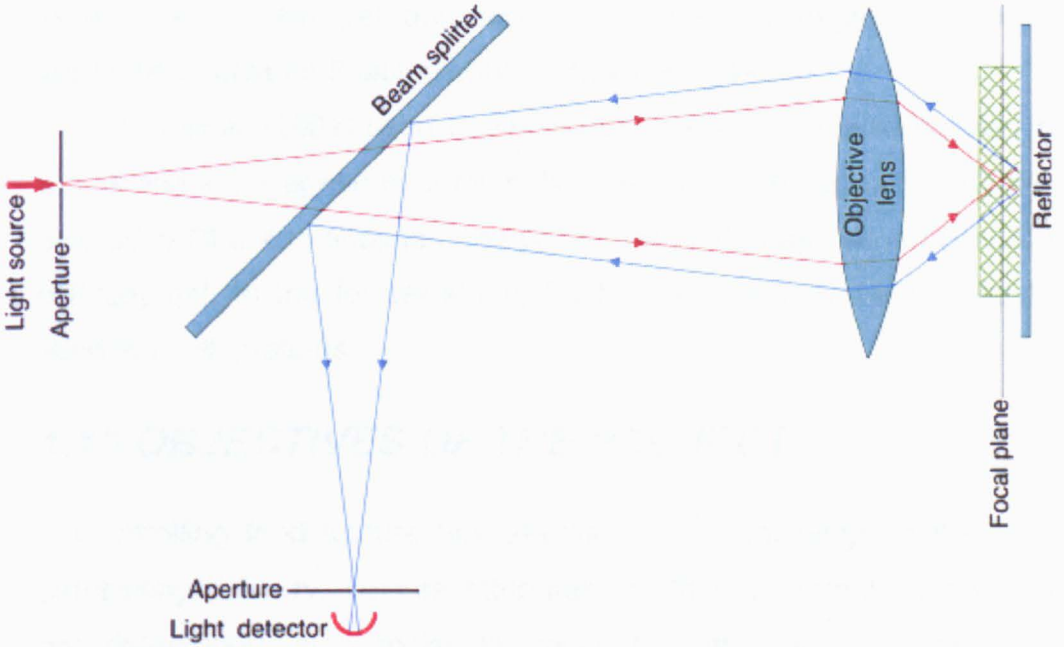
1.9.2 Procedure

The CLSM procedure involves the following steps:

1. Laser light of a specific wavelength emanates from a pinhole.
2. The light is focussed onto a point in the sample where it excites the fluorescent molecules.
3. The excited molecules re-emit a mixture of fluorescent and laser light at different wavelengths.
4. A beam splitter separates the light mixture so that only laser light is allowed to pass through.
5. The fluorescent light is focussed by the same lens onto a pinhole detector.
6. The focal plane of the instrument is then moved by steps of a defined distance so that stacks of optimal sections are recorded.
7. Computer controlled CLSM produces digital images that are then analysed and processed to compute either surface or volume rendered 3D reconstructions of the specimen.

Figure 1.9-A

Schematic Diagram of CLSM Set-up



Adapted from www.upload.wikimedia.org/.../Confocalprinciple.png

1.9.3 Use Of CLSM In Food Materials

The use of CSLM for evaluating the microstructure of milk gels has been reported in quite a few studies. (Lucey & Singh, 1998). CLSM has also been used to quantify the specific changes in the fractal structure of rennet induced caseinate gels (Mellema *et al.*, 2000) In this study, the fractal dimensionality, lower cut-off length and apparent pore size were calculated from the microscopic data and Rhodamine B was used as a label in the milk.

In the study carried out by Durrenberger *et al.* (2001) yam parenchyma was stained with Acid Fuchsin and wheat protein was stained with Safranin O. The authors also reported on the observation of phase separation in amylose and amylopectin in freshly baked bread by CLSM.

Other studies have been carried out with CLSM for observing the structure, cell size, cell shape, cell adhesion, internal air spaces and pore sizes of apples (Lapsley *et al.*, 1992), grapes (Gray *et al.*, 1999), strawberries (Suutarinen *et al.*, 2000), maize and sorghum starch (Huber & BeMiller, 2000) and cheese (Buffa *et al.*, 2001).

In a recent study carried out by de la Fuente *et al.* (2004), CSLM was used to determine cluster/aggregate size and pore size distribution in a whey protein gel and also to assess the degree of phase separation between β-lactoglobulin and κ-carrageenan.

Hemar *et al.* (2002) used CLSM to demonstrate phase separation in a milk and κ-carrageenan combination gels while Roesch *et al.* (2004) also used CLSM to ascertain the pore sizes and phase separation in a milk/soy gel. In the former study, Fast Green CFC dye was used to stain the milk proteins.

1.10 OBJECTIVES OF THE PROJECT

Controlling food texture has always been a challenge in the food processing industry. Texture attributes are thus important factors and are determined, at a molecular level, by the interactions between proteins, fat, carbohydrates and water in the food matrix (Pereira *et al.*, 2003). The growing demand for food with improved nutritional attributes has forced the food industry to reduce the use of traditional ingredients such as sugar, fat and additives. New approaches are required to modulate the eating quality of food products.

Particularly in the manufacture of *Quorn* products, a wide array of ingredients are required to produce not just a meat-like product of adequate and acceptable fibrosity but one which also has succulence, firmness and good flavour. From product development activities at Marlow Foods, it is known that there is a high correlation between egg albumen/whey protein concentrate gel strength and product fibrosity but this connection has not been quantified. The interest in studying the influences and properties of albumen and whey protein concentrate gels is not just that they of themselves are able to create chewy textures, but because they are able to interact within their environment and contribute to the acceptability of the finished product. Whilst egg albumen and whey protein concentrates are typically used as binders, other ingredients such as flavourings, salts, lipids, hydrocolloids and polysaccharides are required to create an acceptable food product. By

the very nature of food, the physical interactions between the constituents as well as chemical reactions encountered during processing, it is inevitable that there will be interactions between these components. These interactions can cause altered functionality by affecting the conformation and molecular stability of the protein molecules.

In *Quorn* products, the protein gel forms a continuous phase whilst the mycoprotein constitutes the discrete phase. In effect, the mycoprotein based meat-free products are made as a fibre-gel composite in which the properties of the finished product are dependent not just on the manner in which discrete particles are held within a continuous phase, but also on the ratio of the discrete phase to the continuous phase. The molecular structure of the mycoprotein bundles is not changed substantially by normal processing methods. However, the molecular structure of the globular protein gel is affected by the heat processes and will contribute significantly to the organoleptic qualities of the end product.

1.10.1 Hypothesis

It is hypothesized that the use of other ingredients can have an effect on the ability of the egg albumen/whey protein concentrate blend to function to its optimum capacity. Some of the other ingredients enhance gelation and water holding capacity while others are antagonistic. It is therefore of considerable and practical importance to understand the role of ingredient interaction on the functional properties of a heat-denatured albumen/whey protein concentrate gel. It is entirely likely that the mechanism for change has to do with the physical state of the gel at the time of consumption. Various studies, as described in previous sections, have shown that protein gels may be particulate, fine-stranded or indeed, a combination of both. *Quorn* products vary in quality. Although much of this is batch-to-batch variation, there is a general lack of understanding of how much the variables that alter the gel characteristics affect the finished product qualities.

It is intended that this research programme concentrate on these issues. For instance, are changes in the textural characteristics of *Quorn* products, for instance, due to the micro and macro-molecules affecting the kind gel that is formed? Can texture be deliberately manipulated by careful selection of added ingredients and/or processing techniques? What degree of interaction exists between the whey protein concentrate and albumen as well as the other ingredients?

The main objectives of this study are thus as follows:

- Determine the effect of added macro- and micro- molecules on the gel characteristics of egg albumen and whey protein-based gels in a typical meat-free analogue.
- Elucidate the relationship between whey protein concentrate and egg albumen in optimising gel strength.
- Quantify the interaction between the binders (egg albumen and whey protein concentrate) and other ingredients in order to be able to predict product characteristics.
- Characterise the types of bonds in a composite gel system using protein dissolution methods.

It is expected that the achievement of these objectives will lead to the ability to predict the textural characteristics of a composite egg albumen and whey protein concentrate gel in food products requiring gel formation and specifically in mycoprotein-based meat-free products.

CHAPTER 2

MATERIALS AND METHODS

2.1 Raw Materials

- Egg Albumen (87% protein db) supplied by Fiske Foods Ltd, Milton Keynes, UK
- Whey Protein Concentrate Oragel 80 (80% protein db, 4% lactose, 0.9% sodium) was obtained commercially from Armor Proteines Ltd, Saint Brice En Cogles, France
- High methoxyl pectin OH 959ND (72% DE) and low methoxyl pectin MRS 160ND (35% DE) were supplied by Degussa Ltd, Newbury, UK.
- Inulin was supplied by Calleva Food Solutions, Basingstoke, Hants, UK
- Dextrose was supplied by Roquette Ltd, Corby, Northants, UK
- Lactose, Cysteine Hydrochloride, Sodium chloride (NaCl) and Calcium chloride were supplied by IFF Ltd, Haverhill, Suffolk, UK.
- Lecithin (Lecisoy 400) was supplied by Food Ingredient Technology Ltd, Beds, UK
- Soya Isolate EX32 (Protein 86.0% db) was supplied by Solae Ltd, Copenhagen, Denmark
- Rice Starch Remyline AX-DR was supplied by Kreglinger Ltd, Antwerp, Belgium
- Hylon VII (70% amylose corn starch) and Novation 2700 (high amylopectin corn starch, 97% carbohydrate db of which sugars is 2%) were supplied by National Starch Food Innovation Ltd, Manchester, UK
- Methylcellulose SGA50M was supplied by Dow Chemicals Ltd, Middlesex, UK
- Casein EM17 (87% protein db, 0.5% lactose) and Bio-Pure GMP (82.5%protein db, 1.0% lactose) was supplied by Davisco Foods International, Le Sueur, Minnesota, USA

2.2 Chemicals

The following chemicals used in the study were obtained from BDH Ltd, Lutterworth, Leicestershire UK

- Tris (hydroxymethyl) methyl ammonium chloride
- Dithioerythritol (DTE)
- Ethylene diaminetetraacetic disodium salt (EDTA)
- Urea
- Whatman filter paper No 1 (110 mm)
- Sodium dodecyl sulphate (SDS)
- Rhodamine B
- Hydrochloric acid (39% w/v)
- Sodium hydroxide

2.3 Equipment

- UV Spectrophotometer (PU8620, Phillips Research, Redhill, UK)
- Water Bath (Polystat CC1, Huber, Chippenham, UK)
- Centrifuge (Centra MP4, International Equipment Company, Needham, Mass, USA)
- Cellulose casing (Viskase GmBH, Dormagen, Germany)
- High shear mixer (Model L4RT Silverson Machines Ltd, Chesham, Bucks, UK)
- Steam oven (Rational Ltd, Luton, UK)
- Texture Analyser TA XT-2 (Stable Micro Systems, Surrey, UK)
- Ceramic hob (Neff T1213 BSH Home Appliances Ltd, Guernsey UK)
- Homogenizer (Ultra Turrax T18 IKA, St Augustin, Germany)
- Confocal laser scanning microscope (DMRXE TCS SP2 Leica Microsystems Heidelberg, Germany).
- Hunter Lab colour meter (Minolta CE Model, York, UK)

2.4 Preparation Of Buffers And Solutions

I. Buffer #1 - 20mM Tris-HCl, 1 mM EDTA, pH 8.0

II. Buffer #2 - Buffer #1 with 2%(w/v) SDS
III. Buffer #3 - Buffer #1 with 2%(w/v) SDS and 0.5 %(w/v) DTE
IV. Buffer #4 - Buffer #1 with 6M Urea
V. Buffer #5 - Buffer #1 with 6M Urea and 0.5 %(w/v) DTE
VI. Buffer #6 - Buffer #1 with 0.5M NaCl

2.5 Gel Preparation

Solutions of protein (16% w/v) and any additions were prepared using deionised water produced by an ion exchange process. In the first set of experiments, the solutions were made up in accordance with the recipe shown in Table 2.5-1 below and in the second set of samples, they were made up in accordance with the format in Tables 2.5-2 & 2.5-3. After initial mixing with a metal fork to wet the powders, the blends were subjected to high shear mixing a lab mixer with a fine emulsor screen head at speed setting 3000 for 120 s or until all the powders were fully dissolved. The pH of the solutions was adjusted accordingly using either 5M NaOH or concentrated sulphuric acid. The protein solutions were poured into 17 mm diameter cellulose casings and tied at both ends. Gelation of the protein samples was induced by heating the filled casings at 100°C in the steam oven for 20 minutes. After steaming, the gels were cooled down rapidly to below in a blast chiller to 5°C and held overnight at this temperature.

Table 2.5-1

Binder samples recipe

Binder Code	Albumen (g)	Whey Protein (g)	De-Ionised Water (g)	pH
100A	64.0	0.0	336	5
66A34W	42.7	21.3	336	5
34A66W	21.3	42.7	336	5
100W	0.0	64.0	336	5
100A	64.0	0.0	336	6
66A34W	42.7	21.3	336	6
34A66W	21.3	42.7	336	6
100W	0.0	64.0	336	6
100A	64.0	0.0	336	7
66A34W	42.7	21.3	336	7
34A66W	21.3	42.7	336	7
100W	0.0	64.0	336	7
100A	64.0	0.0	336	8
66A34W	42.7	21.3	336	8
34A66W	21.3	42.7	336	8
100W	0.0	64.0	336	8

Table 2.5-2

Protein samples preparation design matrix

Added macro-molecule	Ratio of egg albumen to whey protein concentrate (total 16%)		
	1.5 : 1	2.0 : 1	2.5 : 1
0.0%	Sample 1	Sample 2	Sample 3
0.5%	Sample 4	Sample 5	Sample 6
1.0%	Sample 7	Sample 8	Sample 9
1.5%	Sample 10	Sample 11	Sample 12
2.0%	Sample 13	Sample 14	Sample 15

Table 2.5-3

Protein samples formulation

Sample No	Albumen (g)	Whey protein (g)	De-ionised water (g)	Added ingredient# (g)
1	38.4	25.6	336.0	0.0
2	42.6	21.4	336.0	0.0
3	45.8	18.2	336.0	0.0
4	38.4	25.6	336.0	2.0
5	42.6	21.4	336.0	2.0
6	45.8	18.2	336.0	2.0
7	38.4	25.6	336.0	4.0
8	42.6	21.4	336.0	4.0
9	45.8	18.2	336.0	4.0
10	38.4	25.6	336.0	6.0
11	42.6	21.4	336.0	6.0
12	45.8	18.2	336.0	6.0
13	38.4	25.6	336.0	8.0
14	42.6	21.4	336.0	8.0
15	45.8	18.2	336.0	8.0

* All samples were prepared at pH 5 and pH 6 using either 37% Hydrochloric acid or 5M NaOH.

The following compounds were assessed following the pattern described in the table above

- Inulin
- High Methoxyl pectin
- Low methoxyl pectin
- Lactose
- Dextrose
- Methocel SG A50M
- Hydrogen Peroxide
- Cysteine Hydrochloride
- Soya Protein Isolate
- Calcium chloride
- Lecithin
- Oil
- Glycomacropeptide
- Rice Starch
- Hylon VII (high amylose)
- Novation 2700 (high amylopectin)

2.6 Instrumental Texture Profile Analysis

A two-cycle uniaxial compression – relaxation test sequence was performed using the texture analyser with a 25 kg load cell. The load cell was calibrated each time with a 5 kg weight. The equipment was set to zero automatically by lowering the plate until the bottom surface of the plate just contacted the table. Cylindrical gels sections (17 mm diameter) were cut into pieces of 25mm high (n =5 from each log) using

an adapted cheese cutter and were compressed between the stationary bottom platform and a moving upper plate of the texture analyser. The parameter settings for the TPA test profile were programmed as shown in Table 2.6-1.

Table 2.6-1

Settings for TPA test using SMS Texture Analyser TA XT-2

Parameter	Setting
Test mode set to	**T.P.A**
Probe	**Aluminium SMSP/75**
Trigger force	**5.0 g**
Pre test speed	**10.0.0 mm/s**
Test speed	**2.0 mm/s**
Relaxation time	**5.0 s**
Compression depth	**22.5 mm (90% deformation)**
Upward stroke speed	**2.0 mm/s**

The parameter settings and the operation of the Texture Analyser instrument were automatically controlled through the computer with Texture Expert software version 2.54. The curves of force versus time provided direct and derived objective TPA measurements as described in Section 1.7.4.1. (See Appendix for typical curve)

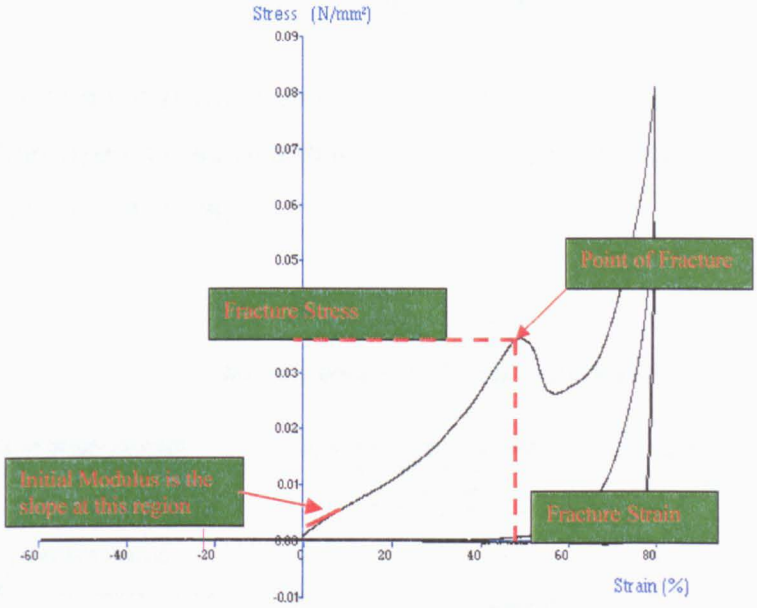
2.6.1 Modified TPA stress-strain curve

The modified TPA curve was used to measure or calculate additional attributes that could not be done using the basic TPA plot. The modification was accomplished by using the software to convert the plot from a force–time XY plot to a stress–strain XY plot. A pictorial representation of the modified TPA plot is shown in Fig. 2.6.A.

Initial modulus was calculated from the modified plot by measuring the slope of the linear section of the plot within the first six seconds of the compression cycle corresponding to a strain value of less than 20% (Fig. 2.6.A). Fracture stress and fracture strain were measured directly from the compression cycle y- and x-axis respectively at the point of fracture (Fig 2.6.A).

Figure 2.6-A

Pictorial Illustration of Modified Stress-Strain TPA Curve



2.7 Stress Relaxation

Using the Texture Analyser, the cylindrical gel sections (17 mm diameter x 25mm high) were compressed to a fixed load of 1500 g and held for 150 s. The parameter settings for the TPA test profile were programmed as shown in Table 2.7-1.

Table 2.7-1

Settings for stress relaxation test using SMS Texture Analyser TA XT-2

Parameter	Setting
Test mode set to	HLDD Force relaxation
Probe	Aluminium SMSP/75
Trigger force	5.0 g
Pre test speed	10.0 mm/s
Test speed	2.0 mm/s
Compression depth	22.5 mm (90% deformation)
Post test speed	10.0 mm/s

Calibration of the texture analyser was carried out as described for the TPA test in section 2.6. Force relaxation was monitored as a function of time. The stress-relaxation curves obtained were evaluated according to the analysis by Peleg (1979; 1980) and the *a*-value

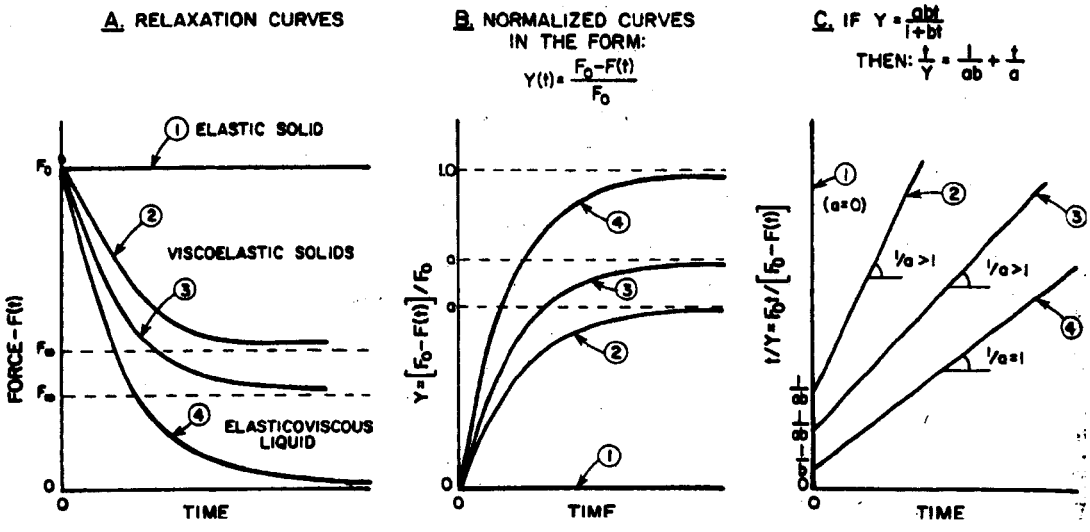
(residual stress) and *b*-value (relaxation time) were obtained as responses. Peleg's constants are shown by the following equation

$$\frac{t}{Y(t)} = \frac{1}{ab} + \frac{t}{a}$$

The *a*-value and the *b*-value were derived as direct measurements from the linearized stress relaxation plot as shown in Fig. 2.7-A. (See Appendix for typical plots)

Figure 2.7-A

Measurement of Peleg's Constants



Adapted from Peleg, 1979

2.8 Expressible Water

Expressible water was evaluated as follows. Filter paper was placed above and beneath the gel during the stress relaxation test. The change in weight of the paper was then measured. The increase in weight of the filter paper from expressed water is used to calculate the loss in weight from the gel plug (17 mm diameter x 25mm high; n =5 from each log).

2.9 Water Uptake

Gel plugs of known weight were immersed in boiling water in an aluminium pan for 120 s. The pan of water was maintained at constant

conditions on the ceramic hob at setting 6. After 120 s, the plugs were patted dry with blotting paper and the change in weight was measured. Water uptake was calculated as the percentage change in weight.

2.10 Colour

Colour was measured using the Minolta Hunter lab. Calibration was carried out following the manufacturer's instructions using the black and white colour standardized tiles. Five individual readings were taken for L^* dimension (variation of colour from white to black) a dimension (variation of colour from red to green) and the b dimension (variation of colour from yellow to blue) from each sample using the automatic measuring system. The geometric mean was calculated.

2.11 Statistical Analysis

For all the tests described in sections 2.6 – 2.9 above, five replicate measurements were taken. The five individual data points were subjected to backward regression and the mean was calculated for the three data points that showed the smallest standard deviation. The raw data was then subjected to statistical analysis using Design Expert statistical software version 6 (Stat-Ease Inc, Minneapolis, USA) in which the variability in the data was partitioned. The statistical significance of each response was tested. It was accomplished by comparing the mean square with the estimated error to yield the p-value for both the main variable and any interactions between the variables. An assessment was then made as to whether the p-value was statistically significant or not.

2.12 Protein Dissolution Experiments

2.5 g of the gelled protein sample was dispersed in 25 ml each of the six buffer solutions in a test tube. The samples were homogenized at room temperature (Ultra Turrax) for 2 min. At the same time, non-gelled samples were also prepared. All the samples i.e. heat gelled and non-heat gelled, were incubated for 1 hour in the water bath at 40°C.

The dispersed samples were centrifuged at 3000 x g for 20 min at ambient temperature and the supernatants were stored at 5°C until required for UV spectroscopy.

The relative amount of protein in the supernatants was measured in 10 x diluted samples at 280 nm on the UV spectrophotometer. The corresponding buffers were also diluted and used as the reference. The flow cell had a path length of 10mm.

2.13 Confocal Laser Scanning Microscopy

Images were recorded with an upright confocal laser scanning microscope (CLSM). Objective lenses 10 × (NA 0.3), 20 × (NA 0.7) and 63 × water-immersion (NA 1.2) were utilized. The samples were mounted on glass slides and protected by cover glass. The protein samples stained with the Rhodamine B were illuminated with GreNe laser at an excitation wavelength of 543 nm. The bandwidth for recording the emission of the fluorescent beams was set from 558 – 700 nm for Rhodamine B. The CLSM was operated in fluorescence mode. The images were recorded at the resolution of 1024 × 1024 and 512 × 512 pixels. In the images, the protein molecules and aggregates appeared as bright patches while the water/non-protein phase appeared dark.

2.13.1 Pre-gelled samples

Samples of protein gels were prepared as described in section gel preparation section above. However, prior to the heat-induced gelation of the sample, the protein was stained by adding Rhodamine B to the protein mixture. The dye/protein ratio was 1:2000. The dye binds non-covalently to the protein network (Alting *et al.*, 2003). Following gelation and blast chilling as described in Section 2.5, the samples were cut into thin slices using a sharp blade in preparation for viewing under the microscope.

2.13.2 Samples gelled in situ

Samples of protein and added compounds were prepared as described above. The protein was stained with fluorescent dye Rhodamine B, dissolved in water with a dye protein ratio of 1:2000. The protein solutions were put into small metal cylindrical containers of volume approximately 80 μL and covered with a glass slip. The container was then situated on a Linkam PE 94 heating and cooling stage and heated at the rate of 10°C from 20 to 95°C.

CHAPTER 3

THE RELATIONSHIP BETWEEN THE TYPE OF BINDER MAKE-UP AND pH

Summary

A combination of initial exploratory experiments including Texture Profile Analysis (TPA), stress relaxation, expressible moisture and water uptake were applied and are discussed under the broad categories summarized in Table 3.1.1.

Table 3.1-1

Examples of Categories of Response Variables

Category	Typical variable
Elastic Properties	**TPA Hardness, TPA Chewiness, Initial Modulus**
Plastic/Viscous Properties	**TPA Cohesiveness, TPA Springiness**
Microstructure	**Water Uptake, Expressible Water**
Fracture Properties	**Fracture Strain, Fracture Stress**
Stress relaxation	**Residual stress (a-value), Stress relaxation rate**
Optical Properties	**Hunter LAB**

The data shown in this section was subjected to statistical analysis in which the effects of pH (numerical variable with data points 5.0, 6.0, 7.0 and 8.0) and binder type (categorical variable of ratio of whey protein concentrate/albumen) were statistically partitioned and averaged over the data points.

The results are discussed in the context of the experiments performed as described in Section 2. Residual stress (*a*-value) and stress relaxation rate (*b*-value) were measured as described in Section 2.7. Expressible water and water uptake were measured as described in Section 2.8 and 2.9 respectively. All the TPA measurements were obtained from the force-time TPA curve as described in Section 2.6. Fracture stress and fracture strain were measured directly from the modified TPA curve as the intercept of the x- and y-axis respectively at the point of fracture as described in Section 2.6.1. The initial modulus was measured as the slope of the curve in the first six seconds of the

modified TPA curve as described in Section 2.6.1. The colour measurements were done by Hunter LAB as described in section 2.10.

Results and Discussion

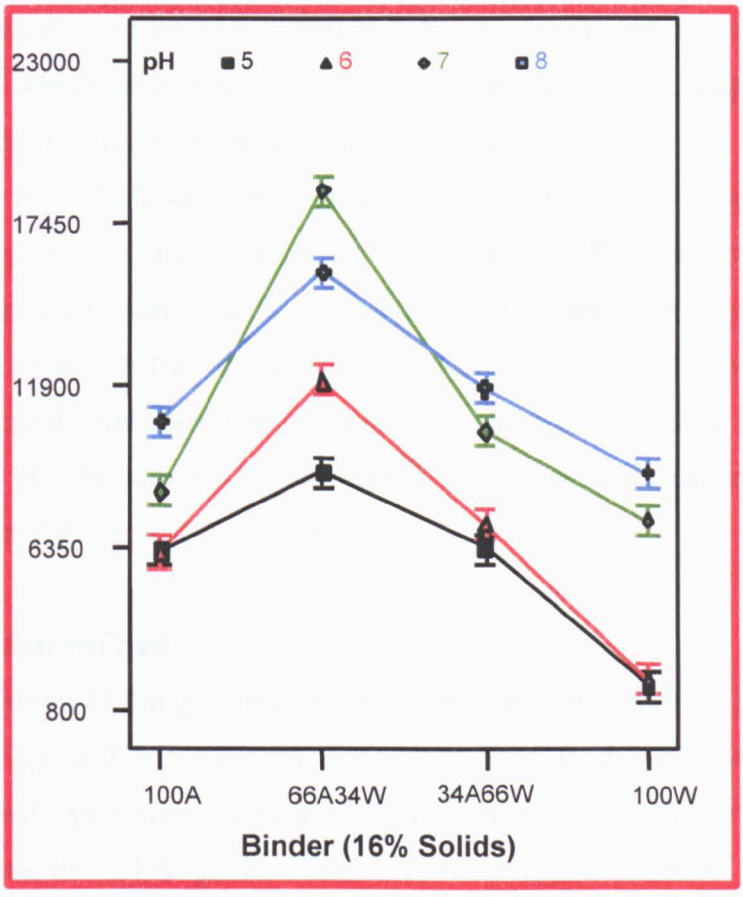
3.1 Texture Profile Analysis

3.1.1 TPA Hardness

Gel hardness is related to the strength of the gel structure and is one of the first and most easily observed texture attribute.

Figure 3.1.1

The Effect of Binder Type and pH on Gel Hardness



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

Figure 3.1.1 shows four different binder mixtures and the effect on the gel hardness as measured by TPA. The maximum hardness

occurred at pH 6.0 with binder 66A34W. On either side of this sample in the plot, the gel was softer.

Another observation from Fig. 3.1.1 was the interaction between pH and the binder composition. It is most evident at pH 7 and 8 where the plots overlap and depending on what binder was used, the hardness value was different. All binder types, with the exception of binder 66A34W, produced harder gels at pH 8. Only with the 66A34W binder was the gel harder at pH 7.0 than it was at pH 8.0. Interestingly, at pH 7.0 and 8.0, the 100W sample was just as hard as the 100A sample. Why? Was this due to extensive unfolding and an enhancement of the thiol→ disulphide reaction?

At the acidic pH region of 5.0 and 6.0, the replacement of as much as 66% of the albumen with whey protein concentrate did not appear to affect the gel hardness significantly. The hardness was of similar magnitude to the 100A sample. Only when the albumen is replaced completely by the whey protein concentrate (100W) did the gel hardness drop significantly. It is most likely that there is a maximum concentration at which the amount of whey protein concentrate in the binder significantly reduces the gel hardness as compared to an all-albumen gel. The maximum concentration will need to be determined for each relevant pH as further work.

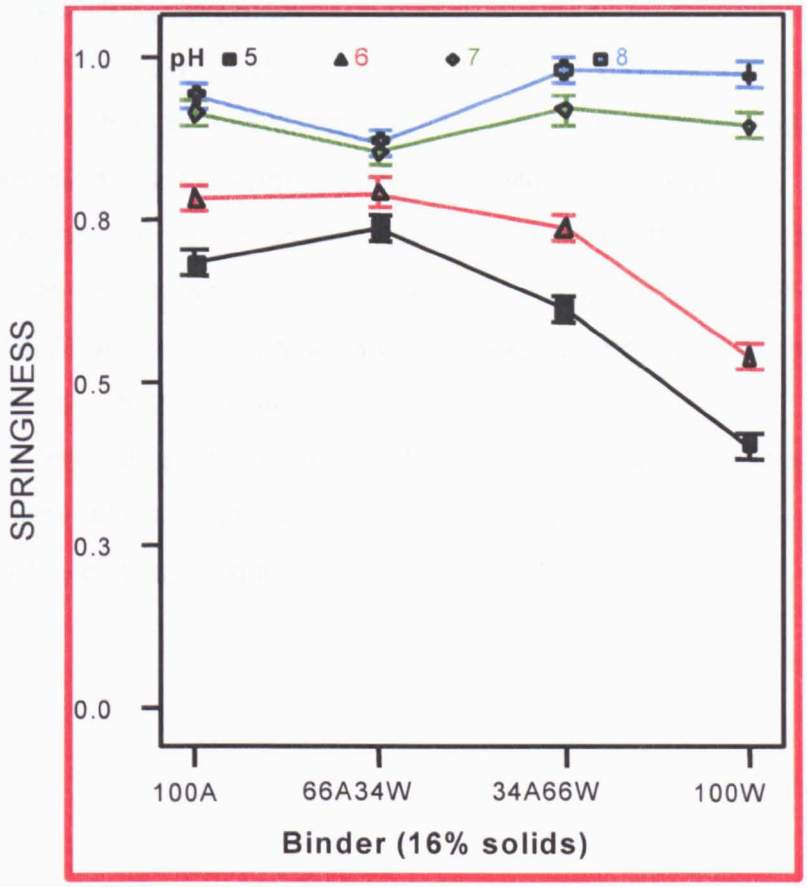
3.1.2 TPA Springiness

The springiness of the gel samples demonstrated an unusual pattern as shown in Figure 3.1.2. At pH values from 5.0-6.0, the 66A34W gel had the highest springiness value of all four binders. However at pH 7.0 and 8.0, the 66A34W gel had the lowest springiness value. In fact there was no significant difference between the 66A34W gel springiness at pH values of 7.0 or 8.0. All four binders exhibited almost the maximum possible springiness at pH 7 and 8. The implication of this phenomenon is that at these high pH values, the gel samples were exhibiting very low viscous properties and were showing elastic behaviour similar to near-perfect solids (Puvanenthiran *et al.*, 2002). As

the pH was reduced further, a higher concentration of whey protein concentrate in the binder led to a reduction in springiness i.e. an increase in the viscous properties. Increased viscous behaviour of a gel is symptomatic of an increase in the effect of water. This suggests that less water is actually physically bound in the gel pores as the pH is reduced to more acidic values.

Figure 3.1.2

The Effect of Binder Type and pH on Gel Springiness



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

Just like the gel hardness, replacement of the albumen with whey protein concentrate at 66% (34A66W) and 100% (100W) did not change the springiness as long as the pH was 7.0 or 8.0. However it would appear that the magnitude of the effect of the whey protein concentrate on springiness was accelerated to larger values as the pH

was reduced further. The same is true at 34% replacement (34A66W). The gel springiness at pH 6 was not significantly differently from the 66% (66A34W) but at pH 5.0, the springiness of the gel made with the 34A66W binder was lower than the gel made from the 66A34W binder. Whether the change progresses further or stabilizes as the pH is lowered further away from the isoelectric point is unknown at this stage.

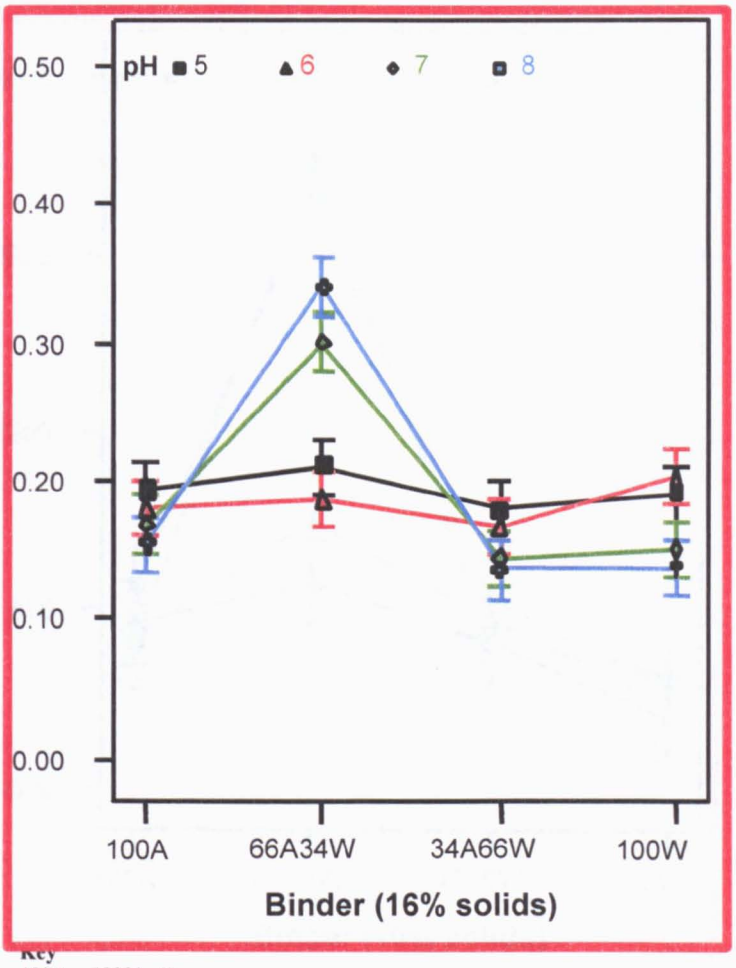
3.1.3 TPA Cohesiveness

Figure 3.1.3 shows that the cohesiveness of the gel samples was maximal with the 66A34W sample. At all other binder ratios, the cohesiveness did not change significantly. Moreover, at pH 5.0 and 6.0 there was no difference in the cohesiveness of the gel samples. However, at the neutral and alkaline pH values, the cohesiveness of the 66A34W sample was significantly increased. Other binder ratios did not appear to exhibit any effects as the pH was altered.

Neither the cohesiveness of the 100A sample nor that of the 100W samples was affected at all by changes in the pH from 5.0 – 8.0 as shown by the overlapping error bars in Fig. 3.1.3.

Figure 3.1.3

The Effect of Binder Type and pH on Gel Cohesiveness



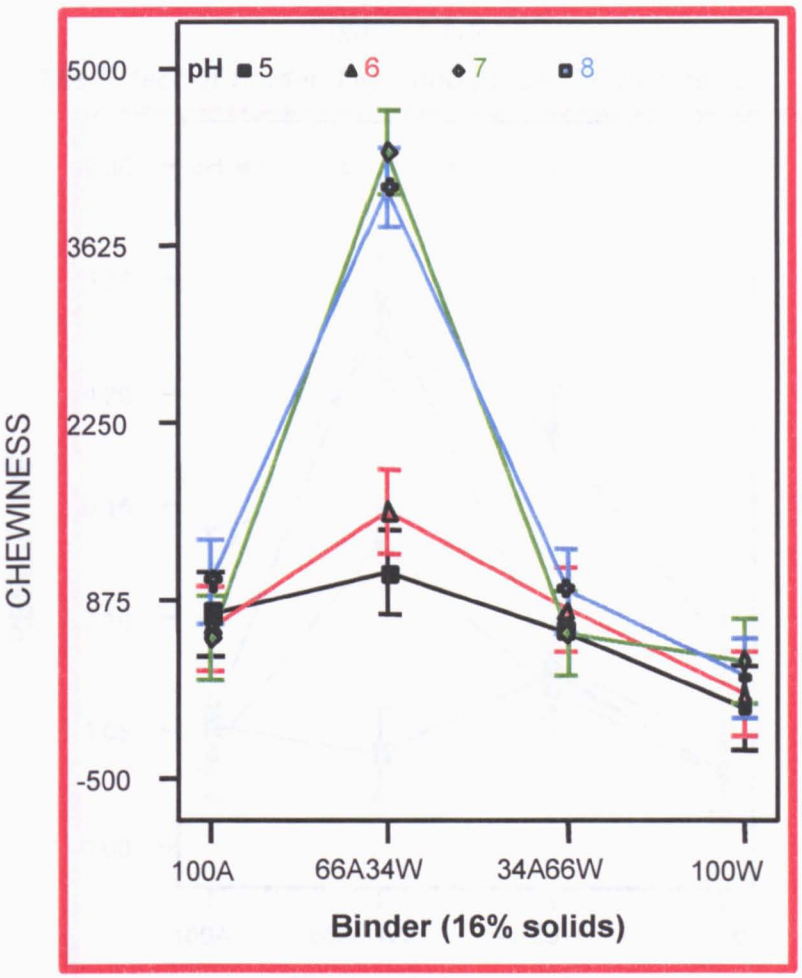
Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

3.1.4 TPA Chewiness

The overall pattern of the chewiness of the gel samples was very similar to cohesiveness (Fig. 3.1.4) and the two exhibit a high degree of correlation. The effect of the binder type on chewiness was maximal with the binder 66A34W. This was particularly evident at pH 7.0 and pH 8.0 where the gel chewiness was several times the value of the other binder mixes.

Figure 3.1.4

The Effect of Binder Type and pH on Gel Chewiness



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

Another noteworthy observation was that the chewiness of the 100A and the 100W samples were not significantly different from each other at any of the pH values. Especially in the acidic pH region, the addition of whey protein to the albumen-based binder system (34A66W) did not change the gel chewiness.

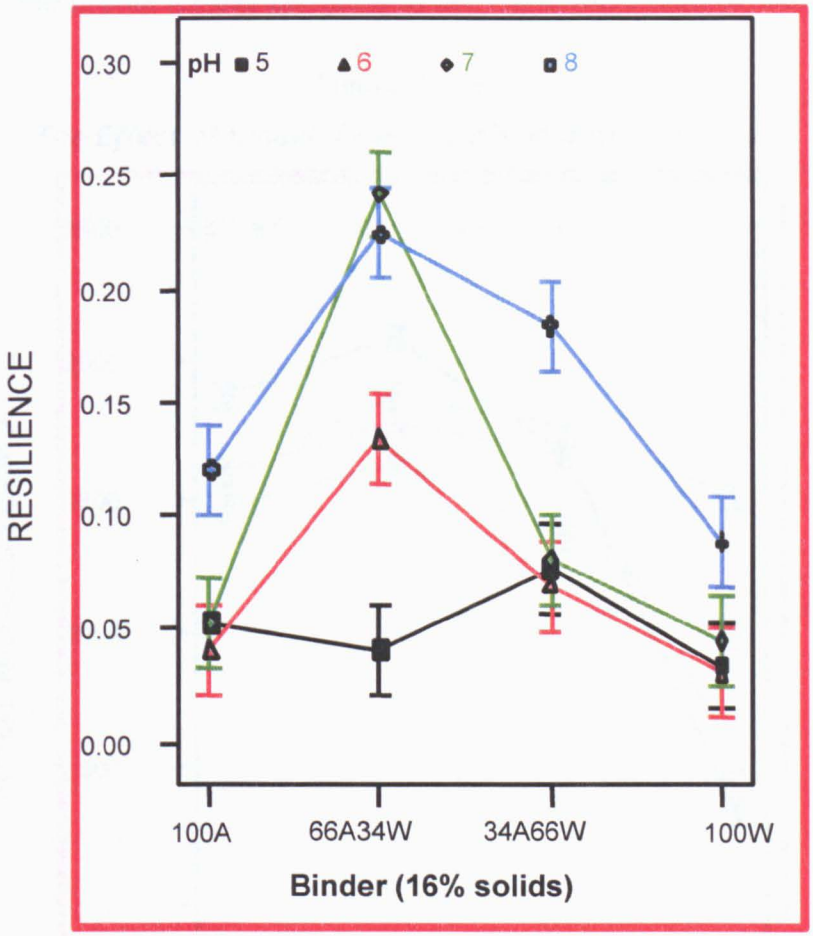
3.1.5 TPA Resilience

At pH values of 6.0 and above, the 66A34W gel was the most resilient of all the binders for each pH group (Fig. 3.1.5). However at

pH 5.0, the resilience of the 66A34W gel was the same as that of the other binders and no significant differences were noted.

Figure 3.1.5

The Effect of Binder Type and pH on Gel Resilience



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

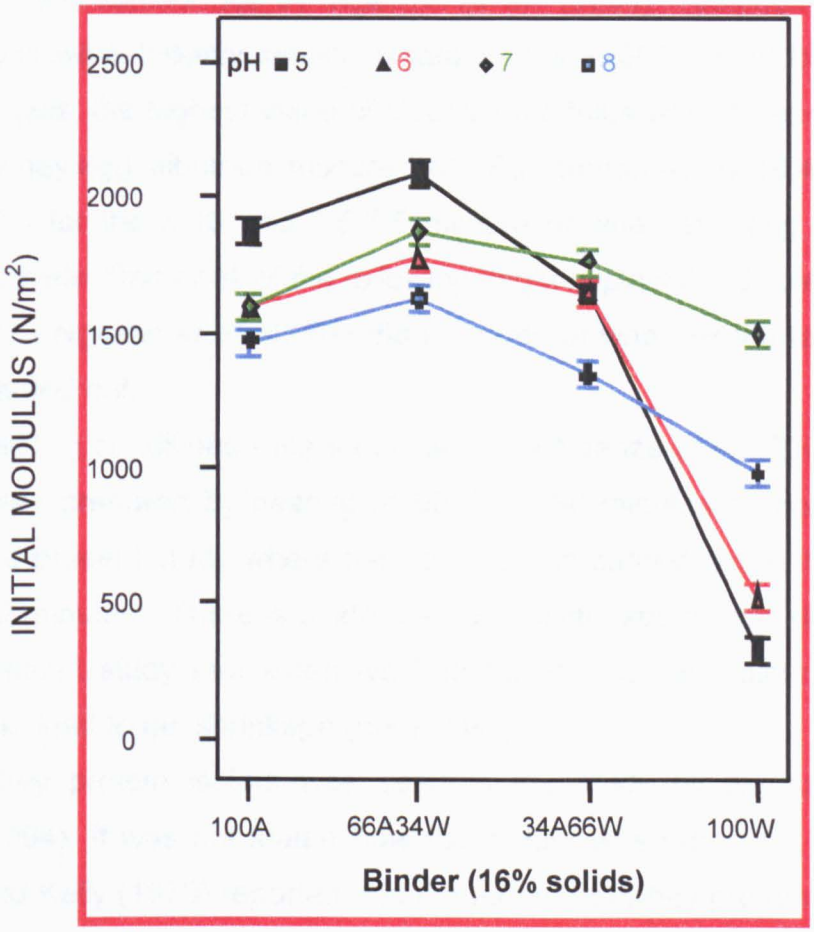
The TPA resilience was significantly lower for the 100A and the 100W binder as compared to the 66A34W at all pH values except pH 5.0. In actual fact, at pH 6.0 and 7.0 the resilience of the binder samples 100A, 34A66W and 100W was insensitive to pH. Only at the pH 8.0 were there any differences in resilience between these three samples. It is clear that there was some synergism between the ratio of whey protein concentrate and albumen and its effect on the resilience.

3.1.6 Initial Modulus

As the pH was raised from 5.0 to 8.0, the initial modulus of all the samples declined as seen in Figure 3.1.6. The profiles of the plots for pH values in the acidic region (pH 5 & 6) were similar. In the same manner, the profile for the samples at pH 7 & 8 were also similar.

Figure 3.1.6

The Effect of Binder Type and pH on Initial Modulus



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

It is to be noted though that at each pH category evaluated, the 66A34W binder was generally the sample with the highest initial modulus. At all four pH values, the 66A34W sample actually had a significantly higher initial modulus than the 100A sample. The result showed that the introduction of whey protein concentrate to an

albumen binder system conferred increased elasticity to the overall gel. **This is a major observation not previously reported**. The 100W gel was the sample most significantly affected by pH as shown by the high degree of separation between the plots. It is also the sample in which the acidic pH values had the biggest effect in reducing the initial modulus.

The results for initial modulus in the present test contrast with an experiment carried out by Ngarize *et al.* (2004). Although the test methods were broadly similar, Ngarize *et al.* (2004) found that in the mixed gels, the highest value of Young's modulus was observed with a 10:5 whey/egg albumen mixture (43.3Pa) compared to 19.4 Pa and 26.0 Pa for the 5:10 and 7.5:7.5 mixture of whey and egg albumen respectively. One or all of the following might explain the discrepancy:

I. pH - Ngarize *et al.* (2004) did not state at what pH the tests were carried out.
II. heating conditions - the samples in the Ngarize *et al.* (2004) study were prepared by heating at 90°C for 30 minutes as opposed to the present study where the heating was carried out at 100°C for 20 minutes. There is evidence from initial experiments during the present study that extensive heating of whey and albumen gels can lead to gel shrinkage (not shown).
III. whey protein isolate was used in the study by Ngarize *et al.* (2004). It was not stated how the isolate was produced. Burgess and Kelly (1979) reported that the method of whey protein isolation does affect the gelation depending on the degree of denaturation damage incurred during processing.

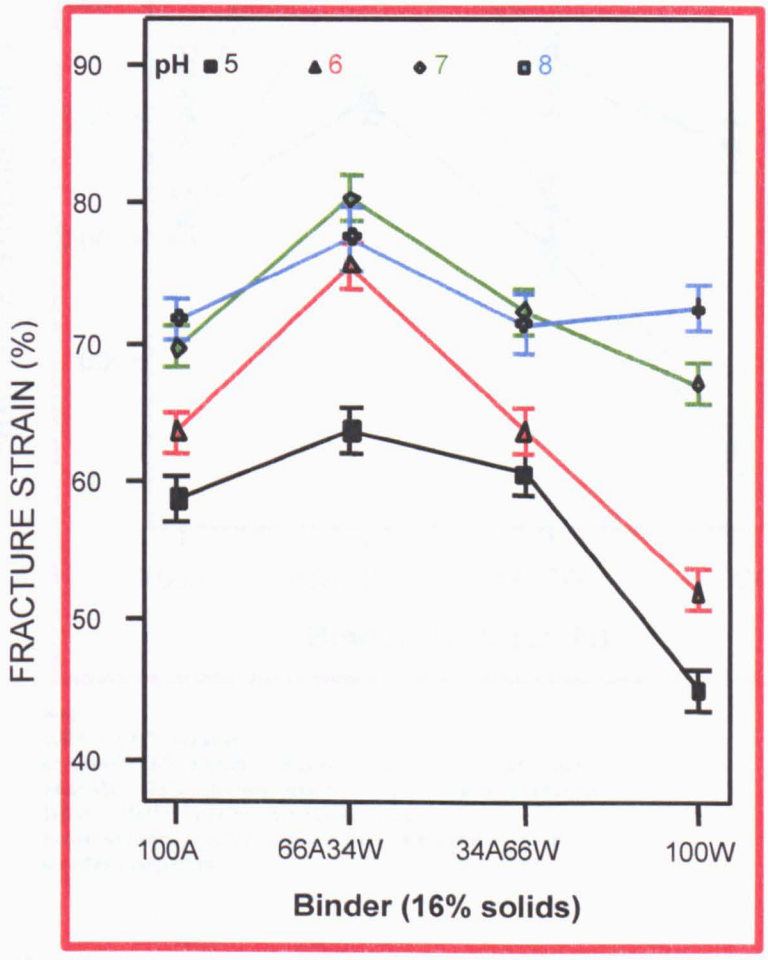
3.1.7 Fracture Strain

As the pH was increased, all the gel samples demonstrated increased fracture strain i.e. increased toughness (Fig. 3.1.7). The increased toughness was particularly evident with the 66A34W sample because at each pH value, the 66A34W was the least brittle i.e. the highest fracture strain of all four binder samples. The 100W binder

was the most significantly affected by the acidic pH. At neutral or alkaline pH values, the 100W gel sample had the same fracture strain value as the 100A binder. The observation demonstrates that in order to maintain the brittleness of an albumen gel in which some of the albumen has been substituted with whey protein concentrate at the same level, a higher pH may be required. This is because at the acidic pH, the 100W fractured at a significantly lower strain value than the 100A and effectively became more brittle. The relationship between the fracture strain of the gel made from 100W binder and pH was not linear.

Figure 3.1.7

The Effect of Binder Type and pH on Fracture Strain



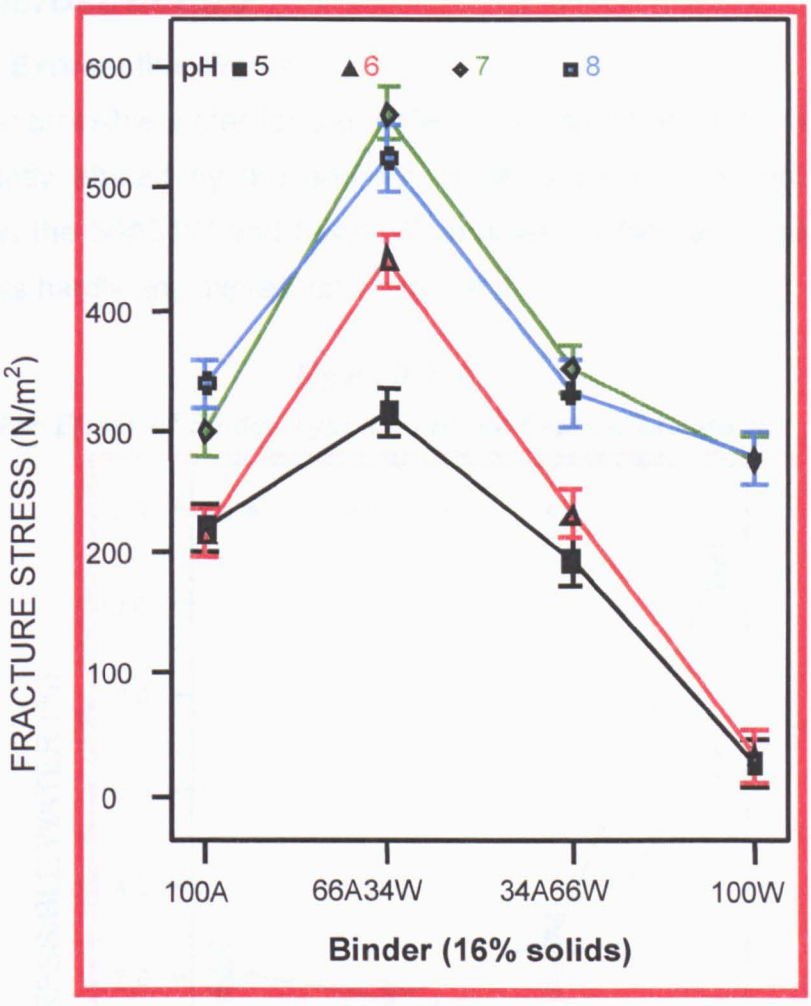
Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

3.1.8 Fracture Stress

The pattern for the fracture stress graph (Fig. 3.1.8) was very similar to fracture strain. The peak for the fracture stress was found in the 66A34W binder for each pH variable.

Figure 3.1.8

The Effect of Binder Type and pH on Fracture Stress



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

For each of the three other binders, there was no significant difference in the fracture stress at pH 7.0 or 8.0. However at pH 5.0 and 6.0, the 100W had a fracture stress value significantly lower than either of the 100A and the 34A66W binder. In fact the fracture stress

value was close to zero. A likely explanation is that the same molecular factors were driving the changes in both the strain at fracture and the stress at fracture. Like the fracture strain, the relationship between the fracture stress of the gel made from the 100W binder and the pH was not linear.

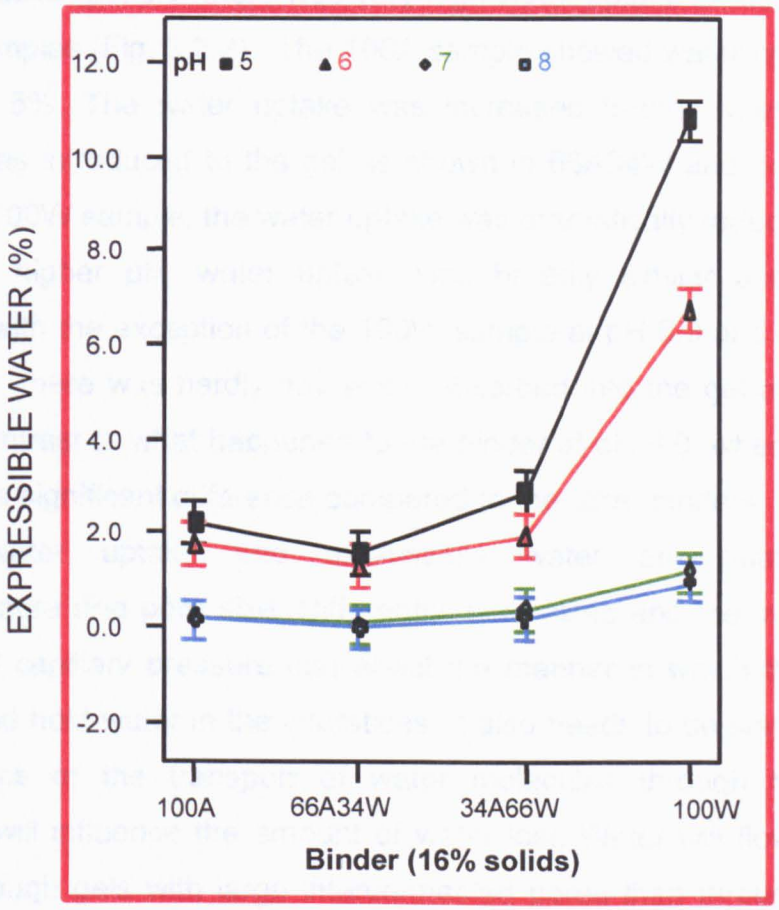
3.2 Microstructure

3.2.1 Expressible Water

The expressible water for the 100A gel sample (Fig. 3.2.1) was not significantly altered by the addition of whey protein concentrate as shown in the 66A34W and 34A66W samples. In fact, at these levels, there was hardly any expressible water at all.

Figure 3.2.1

The Effect of Binder Type and pH on Expressible Water



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

Neutral or alkaline pH appeared to help maintain the integrity of these gel samples and reduced the amount of expressible water. However, the 100W sample was different. At the acidic pH values, the amount of expressible water was considerable. The high expressible water was probably due to the extensive protein aggregation at the low pH. Aggregation of the protein molecules is favoured near the isoelectric point and can lead to tortuous or irregular paths in the gel microstructure (Lucey *et al.,* 1998). Tortuous paths in turn, make gel networks more susceptible to damage when a force is applied and the expressed water cannot easily be re-absorbed when the force is removed (Verheul and Roefs, 1998; Chantrapornchai and McClements, 2002).

3.2.2 Water Uptake

At the acidic pH 5.0, there was very high water uptake across all the binder samples (Fig. 3.2.2). The 100A sample showed water uptake of just over 5%. The water uptake was increased further when whey protein was introduced to the gel as shown in 66A34W and 34A66W. With the 100W sample, the water uptake was dramatically reduced.

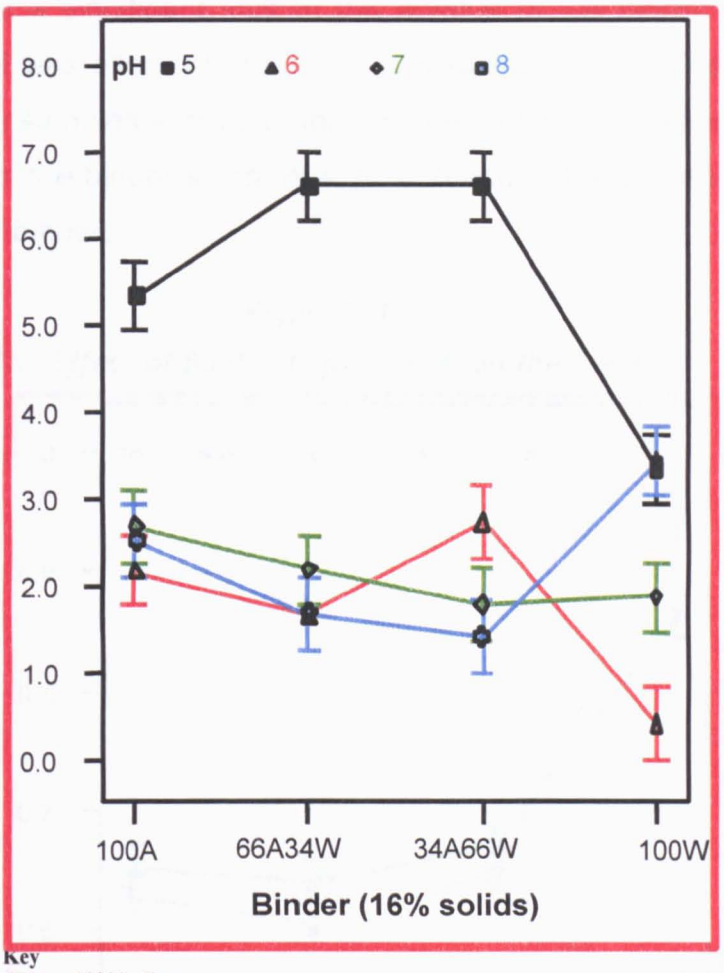
At the higher pH, water uptake was broadly similar across all samples with the exception of the 100W sample at pH 6.0 and pH 8.0. At pH 6.0, there was hardly any water absorbed into the gel and was quite a contrast to what happened to the binder at pH 8.0, where there was also a significant difference compared to the other binders.

The water uptake and expressible water are related to microstructure and pore size. Differential pore sizes and the inversely correlated capillary pressure can affect the manner in which the gels absorb and hold water in the interstices. It also needs to be stated that the kinetics of the transport of water molecules through the gel structure will influence the amount of water lost. Water will flow more easily through gels with large interconnected pores than through gels with a fine stranded network (Chantrapornchai & McClements, 2002). High external pressure such as created by hot, aqueous surrounding

can create a pressure gradient that forces water into or out of the interstices.

Figure 3.2.2

The Effect of Binder Type and pH on Water Uptake



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

3.3 Stress Relaxation

3.3.1 a-Value (Residual Stress)

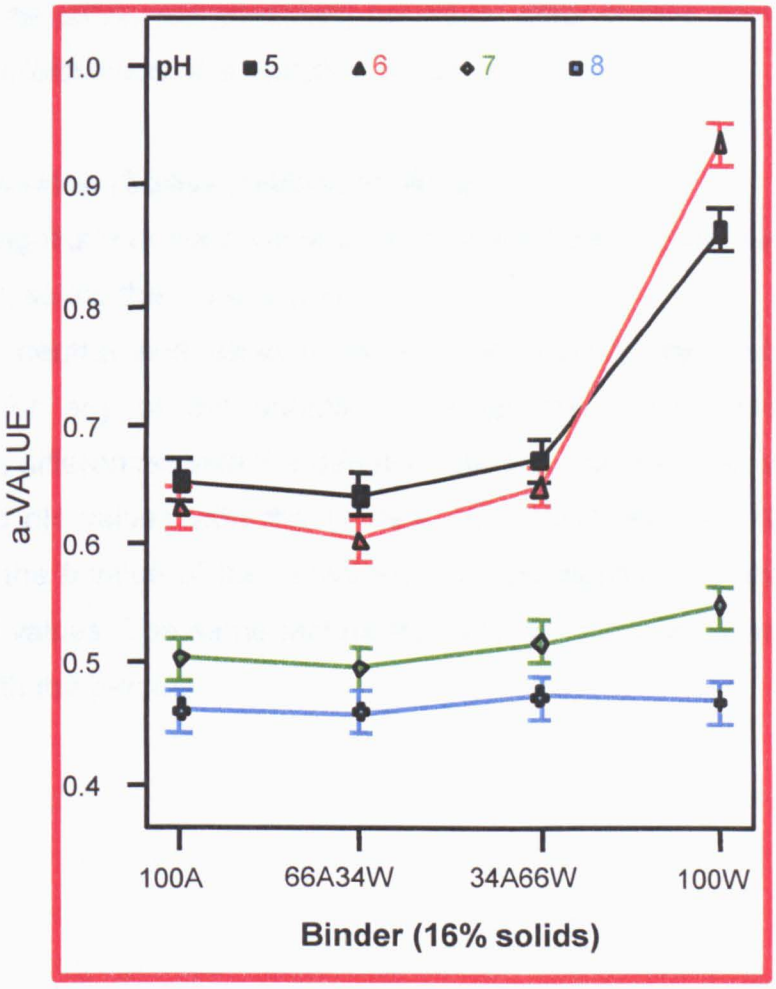
Although very small, there was a significant difference between the *a*-value of all four binder samples that was not affected by either the neutral or alkaline pH (Fig. 3.3.1). There was also a significant, albeit small, difference between the *a*-value of the gels made from the 100A and 100W binder at pH 7.0. When there was a blend of whey protein concentrate and albumen, as evinced by the 66A34W and the 34A66W

samples, the a-value was not affected at either pH 7.0 or 8.0. At pH 5.0 and 6.0, the a-value of the 100W sample was significantly affected at the acidic pH but the other binder mixtures were immune to a change in pH from 5.0 – 6.0.

There was a significant shift in the *a*-value of the binder samples when the pH was altered from acidic to alkaline values. The *a*-value for the binder samples at pH 5.0 and 6.0 was significantly different from the *a*-value of the binder samples at pH 7.0 and 8.0 and even more so for the 100W binder.

Figure 3.3.1

The Effect of Binder Type and pH on the a-value



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

The increase in the a-value of the 100W showed it was demonstrating increasingly viscous behaviour very close to what is predicted for water (1.0). What this likely meant was that much less water was bound up in the gel and as such the 'gel' was behaving like water in its exhibition of strong viscous behaviour. It exhibited less 'solid properties' than the other gel samples. The high expressible water as shown in Fig. 3.1.9 corroborates this explanation. As the pH was increased and more unfolding of the protein molecules took place, more and more water is bound in the interstices and the gel was able to exhibit increased elastic behaviour. It in interesting to speculate whether the performance of the 100W binder would have become more elastic if the water was physically bound by other means with the use of hydrocolloids such as a starch or a gum.

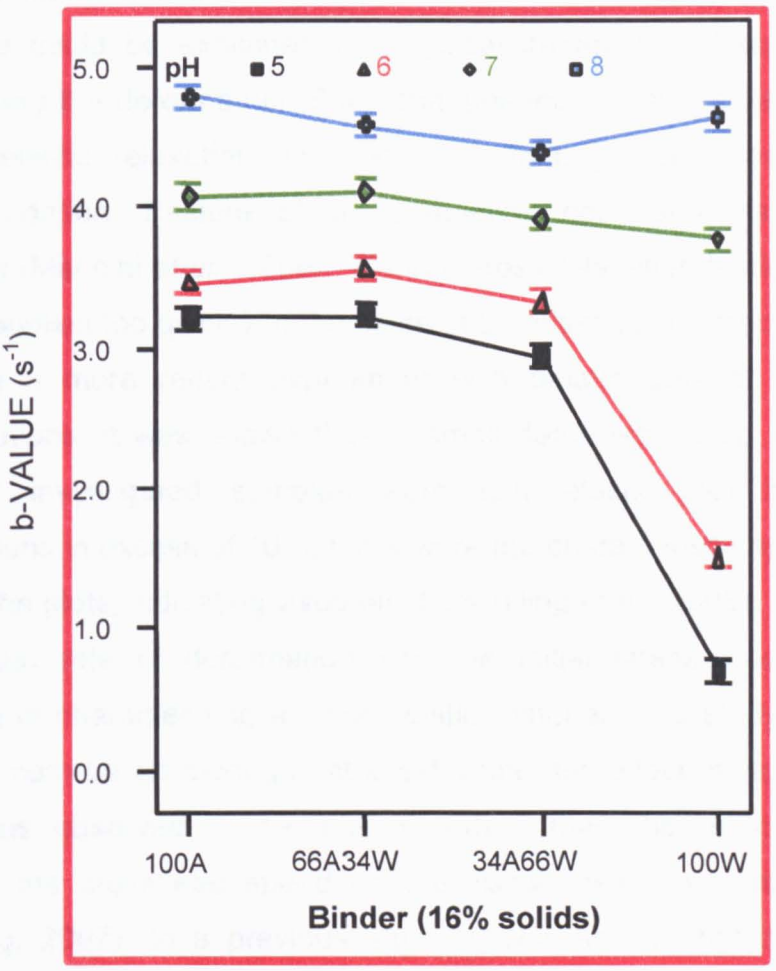
3.3.2 b-value (Stress Relaxation Rate)

The magnitude of the *b*-value clearly followed the pH: as the pH was increased, so did the b-value (Fig. 3.3.2).

At the neutral and alkaline values, the b-value was not greatly affected for any of the samples although there were statistically significant differences with the differing binders. The same was true for the acidic pH values with the binders 100A, 66A34W and 34A66W. However the *b*-value of the 100W sample was significantly reduced at acidic pH values. The same factors that affected the *a*-value were also at play with the *b*-value.

Figure 3.3.2

The Effect of Binder Type and pH on the B-value



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

The degree of deformation can have an effect on the residual forces in biological materials, particularly food samples. In general, it is accepted that at small deformation, many gels are linearly elastic and the relationship between stress and strain is fully characterised by Young's modulus. However at large deformation, the relationship between stress and strain becomes non-linear (Peleg & Calzada, 1979; McEvoy *et al.,* 1985). Peleg and Calzada (1979) noted that there were two regions on the force deformation curve: a rate independent region

in the early stages of deformation (up to 5%) followed by a region in which an increase in deformation rate induces an increase in the force. They concluded that the characteristics of the stress relaxation test procedure could be explained by physical irreversible changes that occur during the deformation. Such changes include stress decay due to visco-elastic relaxation, internal hydrostatic pressure leading to water exudation, fracture of air containing cells and fracture of crosslinks (Mancini *et al.,* 1999). During stress relaxation testing, if the strain is applied too quickly and outside of the elastic limit, cracking can occur. In a more recent experiment with gelatin gels at different concentrations, it was shown that at small deformations up to about 10%, all investigated samples were fully elastic, but at large deformations in excess of 10%, there were the characteristic hysteresis loops in the plots, indicating visco-elasticity (Ding *et al.,* 2008).

Both the rate of deformation and the initial stress loading are important in characterising a visco-elastic material. In a study carried out using corn-based biodegradable extrudate, the effect of varying the stress was observed to be less important than the effect of the changing the crosshead speed on the visco-elastic parameters (Lui and Peng, 2007). In a previous study, it was shown that the initial stress loading had little effect on the a-value (residual stress). However, the initial stress had a tendency to affect the b-value: the larger the initial stress, the steeper the relaxation curve (Peleg (1979).

In spite of the fact that stress relaxation testing is affected by uncertainty and discrepancy because of unavoidable stress decay, this simple test can help at characterising the differences in gel parameters or other textural quality parameters especially when the curves are normalized. In a study of different food materials, it was concluded that the test could be equally applied to materials that are regarded as linear or non-linear from a rheological point of view. It was also shown that both small and large deformations could be treated by the same stress relaxation and curve normalization procedure thereby making it possible to compare curves of different materials. Finally it was

concluded that despite the limitations, the visco-elastic parameters, *a* and *b*, were sensitive enough to indicate curve shape changes in foods subjected to different deformation histories (Peleg, 1979). Herrero and Careche (2005) also applied a stress relaxation test to frozen stored hake and reported a 90% correlation with the sensory assessment results.

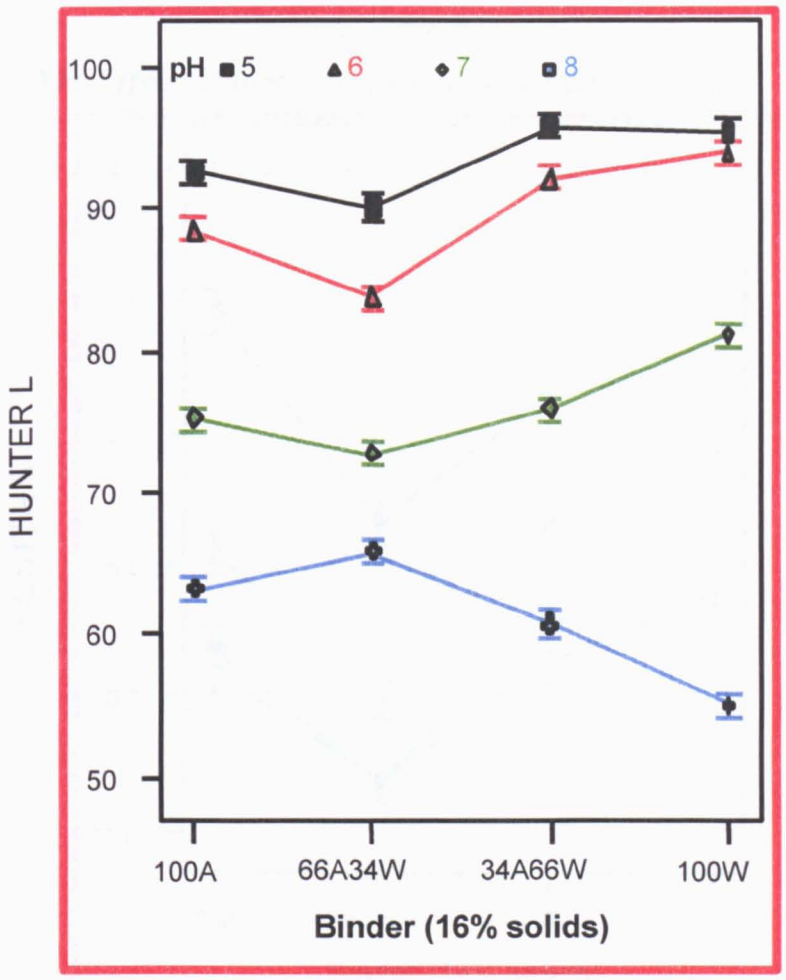
3.4 Optical Properties

3.4.1 Hunter L-value (Lightness)

Both the pH and the type of binder significantly affected the intensity of the gel colour (Fig. 3.4.1). At pH 5.0, 6.0 and 7.0, the 66A34W sample was generally the darkest as shown by the lower Hunter L score in each pH category, with the 100W sample being the lightest. However at pH 8.0 the converse occurred with the 66A34W sample now becoming the lightest and the 100W sample becoming very dark. The reason for the higher L-values is that more light was reflected from the larger aggregates (Resch *et al.*, 2005). Therefore it can be concluded that the Hunter L-value – when there are no other factors at work such as added materials that can influence the colour – is inversely correlated with the aggregate size.

Figure 3.4.1

The Effect of Binder Type and pH on Gel Whiteness



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

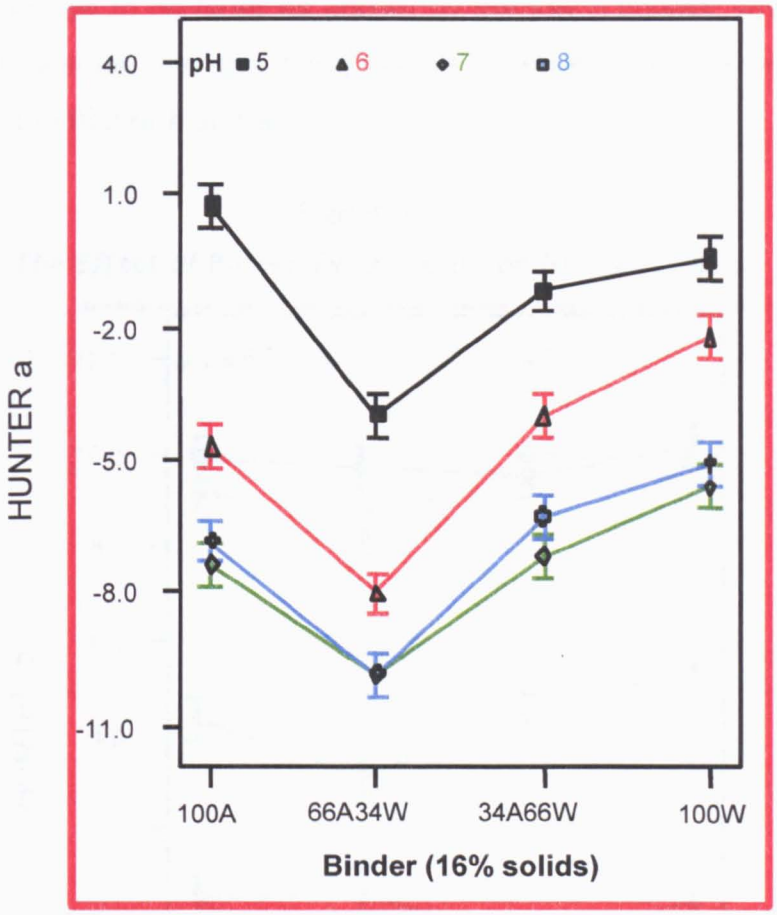
3.4.2 Hunter a-value (red/green chromaticity)

At each pH level, the 66A34W sample had the highest red hue as shown by the low Hunter-a value (Fig. 3.4.2). At pH 5.0, the 100A sample was significantly less red than any other sample (+0.6) but by the time the pH had been adjusted to 6.0, the 100W binder (-1.6) surpassed the 100A binder (-5.2) to become the least red sample at this pH. It was noted that this observation held true for the other pH

values and that only at pH 5.0 was the 100W binder less red than the 100A binder.

Figure 3.4.2

The Effect of Binder Type and pH on Gel Redness



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

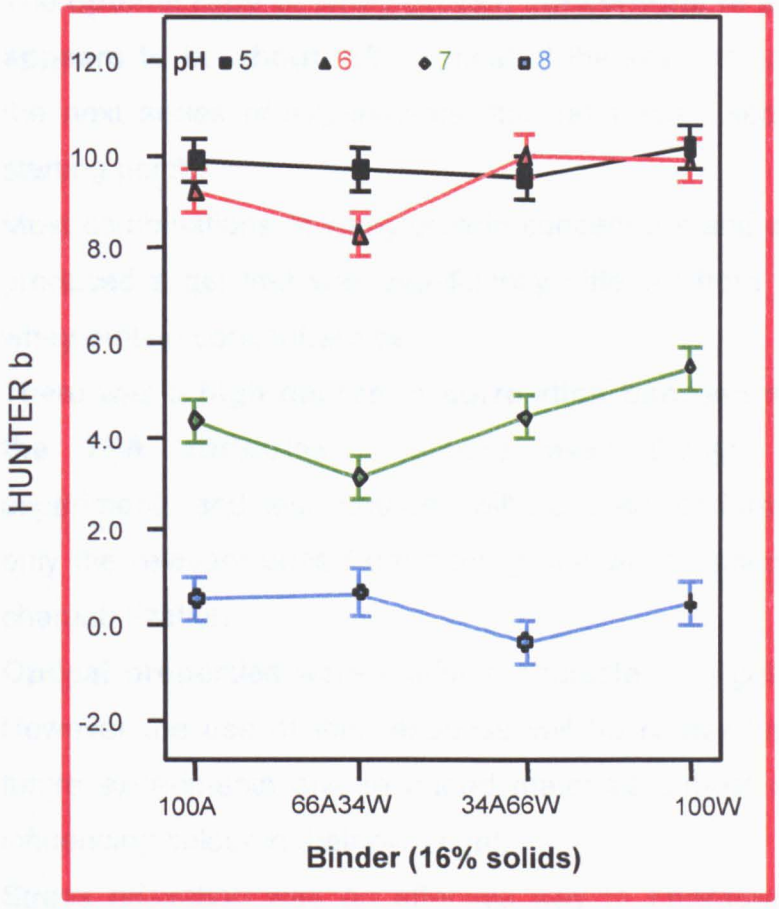
The observations in the present study are in agreement with a theory proposed by Chantrapornchai and McClements (2002) that as the particles become larger, they will become redder (or less green). A reduction in pH closer to the isoelectric point favours aggregation and thus increases the particle size.

3.4.3 Hunter b (yellow/blue chromaticity)

The yellow/green hue did not seem to be significantly affected by the type of binder as shown by the relatively flat lines in Figure 3.4.3. The pH definitely had an effect: at neutral and alkaline values the gel samples tended to be bluer as shown by the low b scores, whereas at the acidic values, the gel samples were more intensely yellow as shown by the higher b scores.

Figure 3.4.3

The Effect of Binder Type and pH on Gel Yellowness



Key
100A = 100% albumen
66A34W= 66% albumen plus 34% whey protein concentrate
34A66W = 34% albumen plus 66% whey protein concentrate
100W = 100% whey protein concentrate
Points are means of 5 replicates. Error bars represent the standard deviation

At pH 7.0, the 100A binder was more yellow than the 100W binder. The observations here are also in agreement with a theory proposed

by Chantrapornchai and McClements (2002) that as the particles become larger, they will become more yellow (or less blue). A reduction in pH closer to the isoelectric point favours aggregation and thus increases the particle size.

3.5 Conclusion/Key Findings

I. Texture Profile Analysis appears to be an ideal test method to characterize the differences between heat-induced gel samples.

II. The **optimal ratio of whey protein concentrate to albumen appears to be about 1:2** for most of the key attributes. For the next series of experiments, this ratio was used as the starting point.

III. Most combinations of whey protein concentrate and albumen produced a gel that was significantly different from a 100% whey protein concentrate gel.

IV. There was a **high degree of correlation between many of the TPA attributes**. Therefore even though all the experiments and test methods will be used for further test, only the relevant ones from each group will be used for gel characterization.

V. **Optical properties** were useful in characterizing gel quality. However the use of this response will be **rather limited** in future experiments due to added materials almost certainly influencing colour in their own right.

VI. Stress relaxation was an effective way to **characterize an potentially partition the viscous and elastic properties of a gel**. It gives an idea as to how much 'unbound' water there is in the gel system and how the water influences the behaviour of the gel system.

CHAPTER 4

THE EFFECT OF VARIOUS MATERIALS AND pH ON GEL CHARACTERISTICS

Summary

The results of the texture analysis of the prepared gels are described. Specific attributes were selected to cover the key categories as follows:

Elastic properties – TPA Hardness

Plastic/Viscous properties – TPA Springiness, Stress relaxation

Microstructure – Water Uptake and Expressible Water

Fracture Properties – Fracture Stress and Fracture Strain

The results are discussed in the context of the experiments performed as described in Section 2. Stress relaxation (a- value) was measured as described in Section 2.7. Expressible water and water uptake were measured as described in Section 2.8 and 2.9 respectively. Hardness and springiness were obtained from the stress-strain TPA curve as described in Section 2.6. Fracture stress and fracture strain were measured directly from the modified TPA curve as the intercept of the x- and y-axis respectively at the point of fracture as described in Section 2.6.1.

The raw data shown in this section were subjected to statistical analysis using Design Expert (Stat-Ease Inc. Minneapolis USA) in which the variability in the responses to the effects of pH (numerical variable with data points 5.0, 6.0, 7.0 and 8.0) and binder type (categorical variable of ratio of whey protein concentrate/albumen) were partitioned. The statistical significance of each response variable was then tested, accomplished by comparing the mean square with the estimated error to yield the *p*-value.

Results and Discussion

4.1 Instrumental Texture Analysis

In this section, the results for the effects of three variables on various texture parameters are shown. The three variables were:

I. binder type (ratio of whey protein concentrate to albumen),
II. pH (values 5.0 and 6.0) and
III. concentration of added material.

4.1.1 Hardness (See Table 4.1.1)

The interaction between pH and the salt concentration was significant for NaCl ($p<0.05$) but not for the $CaCl_2$. However, the pH and concentration of $CaCl_2$ individually have a significant effect on the gel hardness ($p<0.05$).

Table 4.1.1

Analysis of Variance of Factors Affecting Gel Hardness

Compound	Variable	p-value*	Interactions	p-value*	R^2 of model
$CaCl_2$	pH	0.0468	pH:Binder Type	0.0005	0.66
	Binder Type	< 0.0001	pH: Concentration	0.1296	
	Concentration	< 0.0001	Binder Type:Concentration	0.0018	
Casein	pH	< 0.0001	pH:Binder Type	0.5619	0.77
	Binder Type	< 0.0001	pH: Concentration	0.0067	
	Concentration	< 0.0001	Binder Type:Concentration	0.0044	
Cysteine Hydrocloride	pH	0.0001	pH:Binder Type	0.0007	0.82
	Binder Type	< 0.0001	pH: Concentration	0.0610	
	Concentration	< 0.0001	Binder Type:Concentration	0.0062	
Dextrose	pH	< 0.0001	pH:Binder Type	< 0.0001	0.75
	Binder Type	< 0.0001	pH: Concentration	0.0001	
	Concentration	0.0003	Binder Type:Concentration	0.6411	
GMP	pH	< 0.0001	pH:Binder Type	0.0626	0.80
	Binder Type	0.0547	pH: Concentration	0.0013	
	Concentration	< 0.0001	Binder Type:Concentration	0.0138	
High Amylopectin Starch	pH	0.0148	pH:Binder Type	0.0087	0.80
	Binder Type	< 0.0001	pH: Concentration	0.0159	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Amylose Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	0.86
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Methoxyl Pectin	pH	0.2862	pH:Binder Type	0.0014	0.97
	Binder Type	0.2310	pH: Concentration	0.0631	
	Concentration	< 0.0001	Binder Type:Concentration	0.1031	
Hydrogen Peroxide	pH	< 0.0001	pH:Binder Type	0.0018	0.96
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0007	
Inulin	pH	< 0.0001	pH:Binder Type	< 0.0001	0.78
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Lactose	pH	< 0.0001	pH:Binder Type	< 0.0001	0.79
	Binder Type	0.0006	pH: Concentration	< 0.0001	
	Concentration	0.0003	Binder Type:Concentration	< 0.0001	
Lecithin	pH	0.0041	pH:Binder Type	< 0.0001	0.90
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.6749	
Methylcellulose	pH	0.0030	pH:Binder Type	0.0242	0.78
	Binder Type	0.0466	pH: Concentration	0.0013	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
NaCl	pH	0.0901	pH:Binder Type	< 0.0001	0.65
	Binder Type	< 0.0001	pH: Concentration	0.0136	
	Concentration	0.0205	Binder Type:Concentration	0.0001	
Rice Starch	pH	0.0001	pH:Binder Type	< 0.0001	0.82
	Binder Type	0.0007	pH: Concentration	0.0022	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Soya Protein Isolate	pH	0.0015	pH:Binder Type	0.0015	0.79
	Binder Type	0.0063	pH: Concentration	0.2060	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	

** p-values <0.0500 indicate that the model terms are significant*

For the three different starches, there was a significant interaction between all three pairs of variables ($p<0.05$). As it turns out, the effect of each of the individual variables was also significant ($p<0.05$).

In the milk ingredient infused gels, i.e. glycomacropeptide (GMP) and casein, all three main variables individually influenced the gel hardness except for the binder type in the GMP gel, which was involved in a significant interaction with GMP concentration ($p<0.05$).

There was no interaction between the dextrose concentration and binder type although both variables individually affected the hardness.

Gel samples with high methoxyl pectin showed only one paired interaction between pH and binder type ($p<0.01$). The interaction was notable because the individual variables themselves were non-significant. In the case of lecithin, the binder type and lecithin concentration severally had a significant effect on gel hardness but interaction between the two was not significant.

There was no interaction between soya isolate concentration and the pH although individually these two variables affected the hardness. Likewise, the concentration of cysteine hydrochloride did not depend on the pH although the two variables independently had a significant influence on the gel hardness.

4.1.2 Water Uptake (See Table 4.1.2)

The binder type had no effect on the water uptake in the presence of NaCl and was not involved in any interaction with the two other variables. With $CaCl_2$, on the other hand, there was significant interaction between all three pairs of variables ($p<0.001$).

For both the high amylopectin starch and the rice starch, the binder type had no effect on water uptake. However the effect of binder type in the presence of the high amylopectin cannot be discounted because there was a significant interaction observed between the binder type and the pH ($p<0.05$). The same was true of the rice starch where the interaction of binder type and the other two variables was significant ($p<0.001$).

The binder type had no effect on the water uptake in the presence of casein or GMP. However, in both cases, there were significant interactions with at least one of the other variables ($p<0.05$).

Table 4.1.2

Analysis of Variance of Factors Affecting Water Uptake

Compound	Variable	p-value*	Interactions	p-value*	R^2 of model
$CaCl_2$	pH	< 0.0001	pH:Binder Type	0.0009	0.90
	Binder Type	0.0022	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0032	
Casein	pH	< 0.0001	pH:Binder Type	0.0281	0.96
	Binder Type	0.0703	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Cysteine Hydrocloride	pH	< 0.0001	pH:Binder Type	0.1304	0.95
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0046	
Dextrose	pH	< 0.0001	pH:Binder Type	0.0007	0.92
	Binder Type	0.2344	pH: Concentration	0.1319	
	Concentration	0.0199	Binder Type:Concentration	0.0351	
GMP	pH	< 0.0001	pH:Binder Type	0.0060	0.87
	Binder Type	0.0890	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.6075	
High Amylopectin Starch	pH	< 0.0001	pH:Binder Type	0.0445	0.94
	Binder Type	0.1968	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.8258	
High Amylose Starch	pH	< 0.0001	pH:Binder Type	0.0054	0.95
	Binder Type	< 0.0001	pH: Concentration	0.0214	
	Concentration	< 0.0001	Binder Type:Concentration	0.9365	
High Methoxyl Pectin	pH	< 0.0001	pH:Binder Type	0.0446	0.94
	Binder Type	0.2248	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.7441	
Hydrogen Peroxide	pH	< 0.0001	pH:Binder Type	0.1094	0.95
	Binder Type	0.0736	pH: Concentration	0.0014	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Inulin	pH	< 0.0001	pH:Binder Type	0.8360	0.91
	Binder Type	0.0192	pH: Concentration	0.0320	
	Concentration	< 0.0001	Binder Type:Concentration	0.0099	
Lactose	pH	< 0.0001	pH:Binder Type	0.0085	0.94
	Binder Type	0.0250	pH: Concentration	< 0.0001	
	Concentration	0.0003	Binder Type:Concentration	< 0.0001	
Lecithin	pH	< 0.0001	pH:Binder Type	< 0.0001	0.91
	Binder Type	0.0031	pH: Concentration	0.0073	
	Concentration	< 0.0001	Binder Type:Concentration	0.1088	
Methylcellulose	pH	< 0.0001	pH:Binder Type	0.0874	0.98
	Binder Type	0.0428	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
NaCl	pH	< 0.0001	pH:Binder Type	0.0665	0.92
	Binder Type	0.0675	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.2260	
Rice Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	0.99
	Binder Type	0.1371	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Soya Protein Isolate	pH	< 0.0001	pH:Binder Type	0.3310	0.84
	Binder Type	0.2689	pH: Concentration	0.0086	
	Concentration	0.0989	Binder Type:Concentration	0.0705	

** p-values <0.0500 indicate that the model terms are significant*

In the presence of dextrose, the binder type variable had no effect on its own but was involved in significant interactions with the other two variables ($p<0.05$). Owing to this observation, the effect of dextrose cannot be discounted. The opposite was observed for lactose where all three variables showed significant pair-wise interactions ($p<0.01$).

The binder type of itself could not be shown to have an effect on water uptake in the high methoxyl pectin infused gel but was involved in a significant interaction with the pH ($p<0.05$) and thus will always have to be taken into account. With the methylcellulose, lecithin, inulin and cysteine hydrochloride infused gels, all three main variables significantly affected the water uptake ($p<0.05$).

For the soya protein isolate, only the pH, as an independent variable, affected the water uptake. The soya isolate concentration was involved in a significant interaction with the pH ($p<0.01$) and will need to be kept in the model for the purpose of maintaining hierarchy.

4.1.3 **Expressible Water** (See Table 4.1.3)

In the presence of virtually all the ingredients, the three variables exhibited significant interactions with each other in affecting the expressible water. There were only a few exceptions. For the milk-based ingredients and the sugars except for GMP and lactose respectively, there was no interaction between pH and binder type.

The binder type did not affect the expressible water with the cysteine hydrochloride gel but the interaction of binder type with the other two variables was significant ($p<0.01$).

Table 4.1.3

Analysis of Variance of Factors Affecting Expressible Water

Compound	Variable	p-value*	Interactions	p-value*	R^2 of model
$CaCl_2$	pH	< 0.0001	pH:Binder Type	< 0.0001	0.94
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Casein	pH	< 0.0001	pH:Binder Type	< 0.0001	0.99
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0002	
Cysteine Hydrocloride	pH	< 0.0001	pH:Binder Type	0.0001	0.98
	Binder Type	0.3025	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Dextrose	pH	< 0.0001	pH:Binder Type	< 0.0001	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
GMP	pH	< 0.0001	pH:Binder Type	0.0529	0.99
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Amylopectin Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	0.99
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Amylose Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	0.98
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Methoxyl Pectin	pH	< 0.0001	pH:Binder Type	< 0.0001	0.99
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Hydrogen Peroxide	pH	< 0.0001	pH:Binder Type	< 0.0001	0.99
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Inulin	pH	< 0.0001	pH:Binder Type	< 0.0001	0.99
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Lactose	pH	< 0.0001	pH:Binder Type	0.2944	0.94
	Binder Type	0.0057	pH: Concentration	0.0012	
	Concentration	0.0049	Binder Type:Concentration	< 0.0001	
Lecithin	pH	< 0.0001	pH:Binder Type	0.0005	0.99
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Methylcellulose	pH	< 0.0001	pH:Binder Type	0.0035	0.99
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
NaCl	pH	< 0.0001	pH:Binder Type	< 0.0001	0.99
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Rice Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Soya Protein Isolate	pH	< 0.0001	pH:Binder Type	< 0.0001	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	

** p-values <0.0500 indicate that the model terms are significant*

4.1.4 **Cohesiveness** (See Table 4.1.4)

Both salts had highly significant effects on the cohesiveness for all three main variables ($p<0.01$).

In the presence of high amylopectin starch, the effects of the binder type and the concentration of the starch could not be separated as they were both involved in a significant interaction ($p<0.01$). With the two other starches, all the variables individually had a significant effect on the gel cohesiveness ($p<0.01$).

Table 4.1.4

Analysis of Variance of Factors Affecting Cohesiveness

Compound	Variable	p-value*	Interactions	p-value*	R^2 of model
$CaCl_2$	pH	0.0337	pH:Binder Type	< 0.0001	0.75
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Casein	pH	0.0978	pH:Binder Type	0.0024	0.80
	Binder Type	0.0305	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Cysteine Hydrocloride	pH	0.0035	pH:Binder Type	0.0577	0.56
	Binder Type	0.2379	pH: Concentration	0.0005	
	Concentration	< 0.0001	Binder Type:Concentration	0.5823	
Dextrose	pH	0.0052	pH:Binder Type	< 0.0001	0.69
	Binder Type	0.0064	pH: Concentration	< 0.0001	
	Concentration	0.1487	Binder Type:Concentration	0.0001	
GMP	pH	0.0238	pH:Binder Type	0.3384	0.74
	Binder Type	0.0448	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Amylopectin Starch	pH	0.0002	pH:Binder Type	0.2516	0.48
	Binder Type	0.6278	pH: Concentration	0.0723	
	Concentration	0.1743	Binder Type:Concentration	0.0039	
High Amylose Starch	pH	< 0.0001	pH:Binder Type	0.0008	0.74
	Binder Type	0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Methoxyl Pectin	pH	< 0.0001	pH:Binder Type	0.3483	0.95
	Binder Type	0.0004	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0005	
Hydrogen Peroxide	pH	0.0012	pH:Binder Type	0.0752	0.79
	Binder Type	< 0.0001	pH: Concentration	0.0875	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Inulin	pH	0.0325	pH:Binder Type	0.0005	0.64
	Binder Type	0.0019	pH: Concentration	< 0.0001	
	Concentration	0.0062	Binder Type:Concentration	0.0004	
Lactose	pH	0.9053	pH:Binder Type	0.0032	0.74
	Binder Type	< 0.0001	pH: Concentration	0.0006	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Lecithin	pH	< 0.0001	pH:Binder Type	< 0.0001	0.95
	Binder Type	0.0516	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0008	
Methylcellulose	pH	< 0.0001	pH:Binder Type	0.0802	0.82
	Binder Type	0.0026	pH: Concentration	0.0004	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
NaCl	pH	0.0404	pH:Binder Type	< 0.0001	0.69
	Binder Type	0.0110	pH: Concentration	0.0015	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Rice Starch	pH	< 0.0001	pH:Binder Type	0.2100	0.82
	Binder Type	0.0015	pH: Concentration	0.0002	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Soya Protein Isolate	pH	0.0225	pH:Binder Type	< 0.0001	0.84
	Binder Type	0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	

** p-values <0.0500 indicate that the model terms are significant*

With casein, the pH was involved in significant interactions with the other two variables ($p<0.01$), although singularly the pH had no effect. However with GMP, all three variables had individually significant effects ($p<0.05$).

For both sugars, at least one of the independent variables in each experiment was involved in a significant interaction: concentration for dextrose ($p<0.001$) and pH for lactose ($p<0.001$).

Both high methoxyl pectin and methylcellulose expressed significant effects for all the three variables individually ($p<0.01$). The binder type did not seem to be significant with the lecithin-dosed gels. However it was involved in significant interactions with the other two variables ($p<0.001$). With the cysteine hydrochloride gels, the binder type may be eliminated as a key variable in the gel cohesiveness; it did not have a significant effect on its own and was not involved in any significant interaction with either of the other two. However, it is to be noted that the R^2 value for the model was 0.56, showing only moderate correlation. For hydrogen peroxide, all the main variables had a significant effect ($p<0.001$).

4.1.5 **Residual Stress (A-value)** (See Table 4.1.5)

Virtually all of the ingredients tested affected the residual stress. Both the main effects and the interactions were observed to be significant ($p<0.001$). The only non-significant interaction was between pH and binder type in the soya isolate sample ($p>0.05$). For the other ingredients, even when there were non-significant effects of the main variables, they were involved in significant interactions.

Table 4.1.5

Analysis of Variance of Factors Affecting Residual Stress

Compound	Variable	p-value*	Interactions	p-value*	R^2 of model
$CaCl_2$	pH	< 0.0001	pH:Binder Type	< 0.0001	0.98
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0009	
Casein	pH	< 0.0001	pH:Binder Type	0.0246	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Cysteine Hydrocloride	pH	< 0.0001	pH:Binder Type	< 0.0001	1.00
	Binder Type	0.0240	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Dextrose	pH	< 0.0001	pH:Binder Type	0.0010	0.99
	Binder Type	< 0.0001	pH: Concentration	0.0006	
	Concentration	0.0107	Binder Type:Concentration	0.0011	
GMP	pH		pH:Binder Type		
	Binder Type		pH: Concentration		
	Concentration		Binder Type:Concentration		
High Amylopectin Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Amylose Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Methoxyl Pectin	pH	< 0.0001	pH:Binder Type	0.0004	0.94
	Binder Type	0.0004	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Hydrogen Peroxide	pH	< 0.0001	pH:Binder Type	0.0023	0.92
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Inulin	pH	< 0.0001	pH:Binder Type	0.0130	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Lactose	pH	< 0.0001	pH:Binder Type	0.0082	0.95
	Binder Type	0.8215	pH: Concentration	0.0252	
	Concentration	0.0016	Binder Type:Concentration	0.0006	
Lecithin	pH	< 0.0001	pH:Binder Type	0.0453	0.99
	Binder Type	0.0053	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0003	
Methylcellulose	pH	< 0.0001	pH:Binder Type	0.0006	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
NaCl	pH	< 0.0001	pH:Binder Type	0.0013	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Rice Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Soya Protein Isolate	pH	< 0.0001	pH:Binder Type	0.1579	0.98
	Binder Type	< 0.0001	pH: Concentration	0.0251	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	

** p-values <0.0500 indicate that the model terms are significant*

4.1.6 Relaxation Rate (B-value) (See Table 4.1.6)

Both salts showed significant interactions amongst all three variables ($p<0.01$).

The binder type had no effect on its own in the high amylopectin dosed gel but was involved in a significant interaction with the

Table 4.1.6

Analysis of Variance of Factors Affecting Relaxation Rate

Compound	Variable	p-value*	Interactions	p-value*	R^2 of model
$CaCl_2$	pH	< 0.0001	pH:Binder Type	0.0042	0.98
	Binder Type	0.0477	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Casein	pH	< 0.0001	pH:Binder Type	0.1496	0.94
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Cysteine Hydrocloride	pH	< 0.0001	pH:Binder Type	< 0.0001	0.97
	Binder Type	0.0334	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Dextrose	pH	0.0031	pH:Binder Type	0.0263	0.75
	Binder Type	< 0.0001	pH: Concentration	0.0255	
	Concentration	0.1759	Binder Type:Concentration	< 0.0001	
GMP	pH		pH:Binder Type		
	Binder Type		pH: Concentration		
	Concentration		Binder Type:Concentration		
High Amylopectin Starch	pH	< 0.0001	pH:Binder Type	0.2042	0.98
	Binder Type	0.4594	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0150	
High Amylose Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	0.96
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Methoxyl Pectin	pH	0.0260	pH:Binder Type	< 0.0001	0.98
	Binder Type	0.0030	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Hydrogen Peroxide	pH	0.1979	pH:Binder Type	0.0299	0.79
	Binder Type	0.0039	pH: Concentration	0.0219	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Inulin	pH	0.0070	pH:Binder Type	0.4948	0.93
	Binder Type	0.0002	pH: Concentration	0.0215	
	Concentration	< 0.0001	Binder Type:Concentration	0.0014	
Lactose	pH	< 0.0001	pH:Binder Type	< 0.0001	0.83
	Binder Type	< 0.0001	pH: Concentration	0.0048	
	Concentration	< 0.0001	Binder Type:Concentration	0.0001	
Lecithin	pH	0.9599	pH:Binder Type	0.7037	0.87
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Methylcellulose	pH	< 0.0001	pH:Binder Type	< 0.0001	0.98
	Binder Type	0.0656	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
NaCl	pH	< 0.0001	pH:Binder Type	0.0123	0.92
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Rice Starch	pH	< 0.0001	pH:Binder Type	0.0140	0.98
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Soya Protein Isolate	pH	< 0.0001	pH:Binder Type	0.9400	0.75
	Binder Type	0.4181	pH: Concentration	< 0.0001	
	Concentration	0.4967	Binder Type:Concentration	< 0.0001	

** p-values <0.0500 indicate that the model terms are significant*

concentration of the starch ($p<0.05$).

For dextrose, whilst the concentration by itself was of little consequence, the result of dextrose addition was observed in significant interactions with both the other two variables ($p<0.05$). Lactose exhibited significant interactions among all three main variables ($p<0.01$).

In the methylcellulose gel, the binder type interacted significantly with the other two variables ($p<0.001$) although binder type, as a stand alone variable, had no effect. There were also significant interactions ($p<0.05$) between pH and concentration of the lecithin (lecithin gels) and pH with the concentration of hydrogen peroxide and binder type (hydrogen peroxide-dosed gels).

Soya protein-infused gels showed two non-significant main effects– binder type and concentration – but both were separately involved in significant interactions with the pH ($p<0.001$).

4.1.7 Fracture Strain (See Table 4.1.7)

While two of the main effects were clear of any interactions in the NaCl-dosed gel, all three variables were involved in significant interactions in the $CaCl_2$-dosed gel ($p<0.001$). The binder type and concentration of the NaCl demonstrated significant interaction ($p<0.001$).

All three starches and both milk-based ingredients exhibited interactions in all three variables ($p<0.001$), with the only exception being the pH/binder type interaction in the rice starch-dosed gel and the pH/concentration interaction in the GMP-dosed gel.

High methoxyl pectin, methylcellulose, soya isolate, inulin, cysteine hydrochloride and hydrogen peroxide all showed significant effects for the main variables. There were also significant interactions for all three variables except for pH/binder type in methylcellulose and hydrogen peroxide gels.

In the lecithin-dosed gels, the pH and binder type as main effects were individually significant but none of the interactions appeared to be

crucial. It should be noted though that this conclusion might be misleading, as the R^2 for the model is very low at 0.38. There is no explanation for this observation at the present time, other than possibly experimental error.

Table 4.1.7

Analysis of Variance of Factors Affecting Fracture Strain

Compound	Variable	p-value*	Interactions	p-value*	R^2 of model
$CaCl_2$	pH	< 0.0001	pH:Binder Type	< 0.0001	0.96
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Casein	pH	< 0.0001	pH:Binder Type	0.0039	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Cysteine Hydrocloride	pH	0.0003	pH:Binder Type	0.7895	0.65
	Binder Type	0.0005	pH: Concentration	0.0467	
	Concentration	< 0.0001	Binder Type:Concentration	0.7170	
Dextrose	pH	0.0249	pH:Binder Type	0.0641	0.39
	Binder Type	0.0004	pH: Concentration	0.4759	
	Concentration	0.2695	Binder Type:Concentration	0.7149	
GMP	pH	< 0.0001	pH:Binder Type	0.0170	0.95
	Binder Type	< 0.0001	pH: Concentration	0.4488	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Amylopectin Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	0.98
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Amylose Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	0.98
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Methoxyl Pectin	pH	< 0.0001	pH:Binder Type	0.0070	0.98
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Hydrogen Peroxide	pH	0.0004	pH:Binder Type	0.5366	0.96
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Inulin	pH	< 0.0001	pH:Binder Type	< 0.0001	0.94
	Binder Type	0.0039	pH: Concentration	0.0008	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Lactose	pH	< 0.0001	pH:Binder Type	0.1409	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Lecithin	pH	0.0129	pH:Binder Type	0.0807	0.38
	Binder Type	0.0033	pH: Concentration	0.4139	
	Concentration	0.2124	Binder Type:Concentration	0.5065	
Methylcellulose	pH	< 0.0001	pH:Binder Type	0.1136	1.00
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
NaCl	pH	< 0.0001	pH:Binder Type	0.2603	0.92
	Binder Type	< 0.0001	pH: Concentration	0.0695	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Rice Starch	pH	< 0.0001	pH:Binder Type	0.1114	0.96
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Soya Protein Isolate	pH	< 0.0001	pH:Binder Type	0.0092	0.92
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	0.0227	Binder Type:Concentration	< 0.0001	

** p-values <0.0500 indicate that the model terms are significant*

4.1.8 Fracture Stress (See Table 4.1.8)

Gels made with both salts showed major interactions between the variables. $CaCl_2$ influenced interactions between all three pairs of variables, whilst with NaCl, only the pH/binder type did not exhibit any

Table 4.1.8

Analysis of Variance of Factors Affecting Fracture Stress

Compound	Variable	p-value*	Interactions	p-value*	R^2 of model
$CaCl_2$	pH	< 0.0001	pH:Binder Type	0.0045	0.97
	Binder Type	< 0.0001	pH: Concentration	0.0103	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Casein	pH	< 0.0001	pH:Binder Type	0.0031	0.98
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Cysteine Hydrocloride	pH	< 0.0001	pH:Binder Type	0.0009	0.99
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Dextrose	pH	< 0.0001	pH:Binder Type	0.0054	0.90
	Binder Type	< 0.0001	pH: Concentration	0.0004	
	Concentration	0.5389	Binder Type:Concentration	0.0002	
GMP	pH	< 0.0001	pH:Binder Type	0.0023	0.95
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Amylopectin Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	0.97
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Amylose Starch	pH	< 0.0001	pH:Binder Type	0.0032	0.94
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Methoxyl Pectin	pH	0.0183	pH:Binder Type	< 0.0001	0.99
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Hydrogen Peroxide	pH	< 0.0001	pH:Binder Type	0.0004	0.98
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Inulin	pH	< 0.0001	pH:Binder Type	< 0.0001	0.94
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Lactose	pH	< 0.0001	pH:Binder Type	0.0107	0.86
	Binder Type	< 0.0001	pH: Concentration	0.0551	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Lecithin	pH	< 0.0001	pH:Binder Type	0.0003	0.88
	Binder Type	< 0.0001	pH: Concentration	0.0008	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Methylcellulose	pH	< 0.0001	pH:Binder Type	0.0881	0.95
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
NaCl	pH	< 0.0001	pH:Binder Type	0.1988	0.91
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Rice Starch	pH	< 0.0001	pH:Binder Type	0.0694	0.95
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Soya Protein Isolate	pH	< 0.0001	pH:Binder Type	0.0003	0.94
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	

** p-values <0.0500 indicate that the model terms are significant*

interaction.

All three starches and the milk-based ingredients showed similar significant interactions ($p < 0.01$) except the rice starch in which there was no interaction between the pH and the binder type.

The concentration of dextrose, as a variable, did not have a significant effect on the fracture stress on its own, but demonstrated a significant effect in its interactions separately with both the pH and the binder type ($p<0.01$). With lactose, all the main effects were significant ($p<0.001$) and so were the interactions ($p<0.05$) except for the pH and concentration of lactose.

For high methoxyl pectin, methylcellulose, lecithin, soya protein isolate, inulin, cysteine hydrochloride and hydrogen peroxide, all the main variables and the interactions between these were significant ($p<0.05$). The one exception was the pH and binder type in the methylcellulose based gel, which was not significant.

4.1.9 **Springiness** (See Table 4.1.9)

All the main variables and the interactions for both salt-dosed gel systems were significant ($p<0.05$) as were the main effects and interactions for all the starch products except the binder type and concentration of the high amylose starch gel.

The main variables were significant for both milk-based ingredients as well as all the interactions between the variables in the casein. However, with GMP, only the pH/concentration interaction was significant ($p<0.01$).

The sugars exhibited differences. Of the three variables, the dextrose concentration was not significant on its own, but was involved in an interaction with the binder type ($p<0.01$). The other two variables individually showed significant effects. As for the lactose-dosed gels, the binder type, although not significant by itself, was involved in a significant interaction with the concentration ($p<0.001$).

Likewise, the binder type in the pectin-infused gel did not have a significant effect on gel springiness, but was closely and significantly

associated with the other two variables. The only significant interaction in the lecithin gel was between the binder type and lecithin concentration ($p<0.001$), even though the binder type, by itself, did not affect gel springiness. Similarly, although pH showed no effect in the methylcellulose-infused gel, it was involved in a significant interaction with the concentration ($p<0.001$).

The soya isolate, inulin and the hydrogen peroxide infused gels had significant main variables as well as interactions.

Of the three variables in conjunction with cysteine hydrochloride, the binder type did not show any effect but was involved in a major interaction with the concentration ($p<0.01$).

Table 4.1.9

Analysis of Variance of Factors Affecting Springiness

Compound	Variable	p-value*	Interactions	p-value*	R^2 of model
$CaCl_2$	pH	< 0.0001	pH:Binder Type	0.0162	0.85
	Binder Type	0.0074	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0004	
Casein	pH	< 0.0001	pH:Binder Type	< 0.0001	0.97
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Cysteine Hydrocloride	pH	< 0.0001	pH:Binder Type	0.0743	0.93
	Binder Type	0.0910	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0006	
Dextrose	pH	< 0.0001	pH:Binder Type	0.1922	0.91
	Binder Type	0.0003	pH: Concentration	0.0519	
	Concentration	0.1223	Binder Type:Concentration	0.0002	
GMP	pH	< 0.0001	pH:Binder Type	0.0925	0.92
	Binder Type	< 0.0001	pH: Concentration	0.0002	
	Concentration	0.0205	Binder Type:Concentration	0.5013	
High Amylopectin Starch	pH	< 0.0001	pH:Binder Type	0.0006	0.96
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
High Amylose Starch	pH	< 0.0001	pH:Binder Type	0.0104	0.96
	Binder Type	< 0.0001	pH: Concentration	0.0053	
	Concentration	< 0.0001	Binder Type:Concentration	0.2404	
High Methoxyl Pectin	pH	< 0.0001	pH:Binder Type	< 0.0001	0.92
	Binder Type	0.3263	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Hydrogen Peroxide	pH	< 0.0001	pH:Binder Type	0.0392	0.92
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0004	
Inulin	pH	< 0.0001	pH:Binder Type	0.0019	0.90
	Binder Type	0.0027	pH: Concentration	0.0086	
	Concentration	0.0001	Binder Type:Concentration	0.0054	
Lactose	pH	< 0.0001	pH:Binder Type	0.4039	0.86
	Binder Type	0.8148	pH: Concentration	0.0360	
	Concentration	0.0343	Binder Type:Concentration	0.0003	
Lecithin	pH	< 0.0001	pH:Binder Type	0.8639	0.91
	Binder Type	0.2800	pH: Concentration	0.0889	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	
Methylcellulose	pH	< 0.0001	pH:Binder Type	0.1204	0.95
	Binder Type	0.0005	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0008	
NaCl	pH	< 0.0001	pH:Binder Type	< 0.0001	0.96
	Binder Type	0.0008	pH: Concentration	0.0176	
	Concentration	< 0.0001	Binder Type:Concentration	0.0198	
Rice Starch	pH	< 0.0001	pH:Binder Type	< 0.0001	0.97
	Binder Type	< 0.0001	pH: Concentration	< 0.0001	
	Concentration	< 0.0001	Binder Type:Concentration	0.0114	
Soya Protein Isolate	pH	< 0.0001	pH:Binder Type	< 0.0001	0.96
	Binder Type	0.0215	pH: Concentration	0.0124	
	Concentration	< 0.0001	Binder Type:Concentration	< 0.0001	

** p-values <0.0500 indicate that the model terms are significant*

4.2 The Effect Of Dextrose

Figures 4.2: A-F demonstrate very clearly the interaction between the amount of dextrose, pH and the binder ratio on the response variables.

Elastic Properties

In the present study, dextrose levels up to 2% did not change the elastic properties of the gel samples as shown by hardness at pH 5.0 (Fig. 4.2-A). However, there appeared to be a tendency towards overall reduction in gel hardness as the amount of dextrose is increased from 0% to 2% at pH 6.0.

Figure 4.2-A

Effect of Dextrose on Gel Hardness

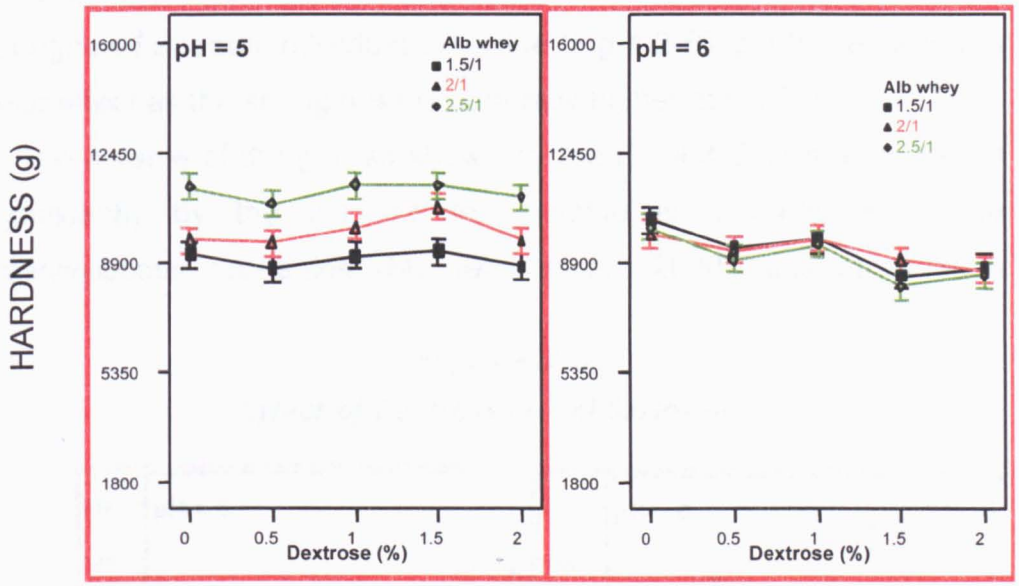


Figure 4.2-A also shows how alteration of the ratio of whey protein concentrate to albumen in the binder changes the elastic properties of the gel. At pH 5.0 and at virtually all levels, increasing the amount of albumen significantly amplified gel hardness ($p<0.05$). The same outcome was not observed at pH 6.0 where changes to the amount of albumen in the binder in the presence of dextrose appeared to have no effect on the hardness.

The normal consequence of adding sugars is to decrease the mechanical properties of a protein gel (Renard *et al.*, 1999). In an experiment carried out by Yamul & Lupano (2003), in which honey was added to a whey protein gel, it was concluded that the honey increased the firmness of the gel especially at low pH values of 3.75 but had no effect on gels prepared at pH 4.2 and 7.0. A previous experiment carried out by Boye & Alli (2000) examined the effect of various sugars on thermal denaturation of whey protein concentrate, although no texture measurements were carried out. The conclusion was that dextrose conferred thermal stability on both the α-lactalbumin and the β-lactoglobulin. The stability was confirmed by an increase in the denaturation temperatures by 1.5°C and 7.7°C respectively.

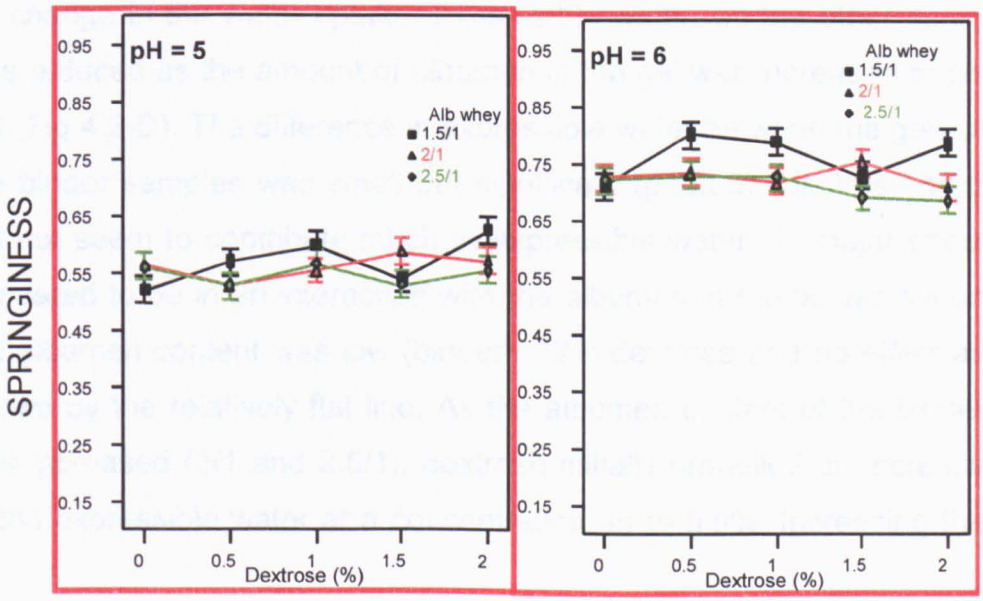
Plastic properties

At up to 2% concentration, dextrose had very little effect on the gel springiness at each individual pH value (Fig 4.2-B). pH however had a clear effect as the springiness is generally higher at pH 6.0.

The *a*-value of the gel, as shown by the Fig 4.3-G was also altered significantly by the pH. As the *a*-value is a measure of the plastic/viscous properties (Messens *et al.*, 2000), this observation

Figure 4.2-B

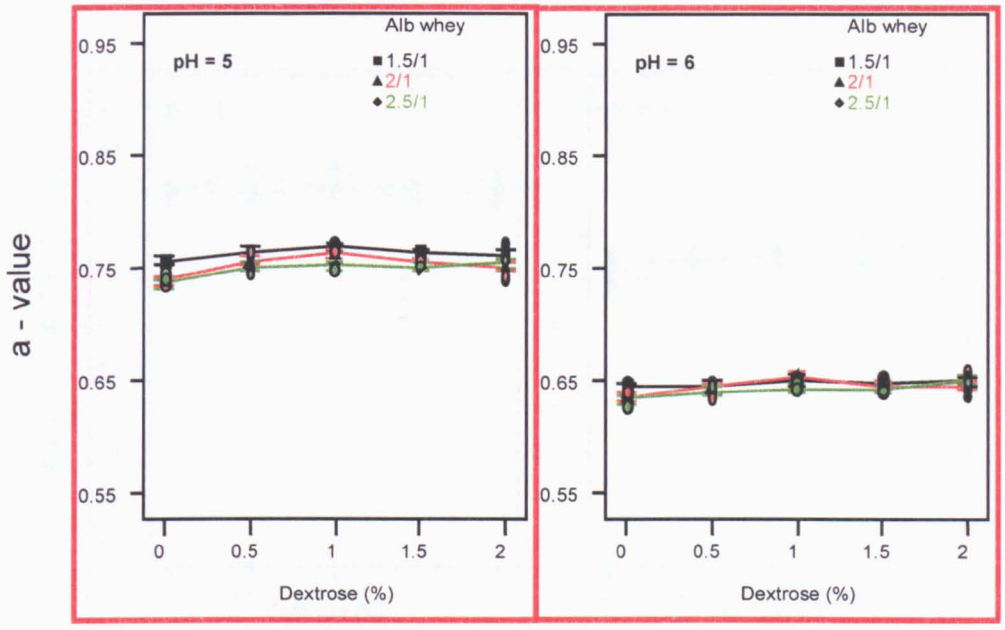
Effect of Dextrose on Gel Springiness



confirmed that the addition of dextrose had no effect on the viscous properties of the binder systems. Neither did the binder type.

Figure 4.2-G

The Effect of Dextrose on a value



Micro-structure

The addition of dextrose did not seem to have much effect on the microstructure of the gels. As shown by Fig. 4.2-C. there was virtually no change in the water uptake. Expressible water, on the other hand, was reduced as the amount of albumen in the gel was increased at pH 5.0 (Fig 4.2-D). The difference in expressible water between the gels of the binder samples was small but significant ($p<0.05$). Dextrose itself did not seem to contribute much to expressible water: its major effect appeared to be in an interaction with the albumen in the binder. When the albumen content was low (binder 1.5/1) dextrose had no effect as shown by the relatively flat line. As the albumen content of the binder was increased (2/1 and 2.5/1), dextrose initially propelled an increase in the expressible water at a concentration up to 0.5%. Increasing the

quantity of dextrose in the gel system led to a small but steady decline at concentrations up to about 1.5 – 2.0%.

At pH 6.0, there was very little change in the expressible water and the ostensible interaction that was described between dextrose concentration and pH value 5.0 was not seen at pH 6.0.

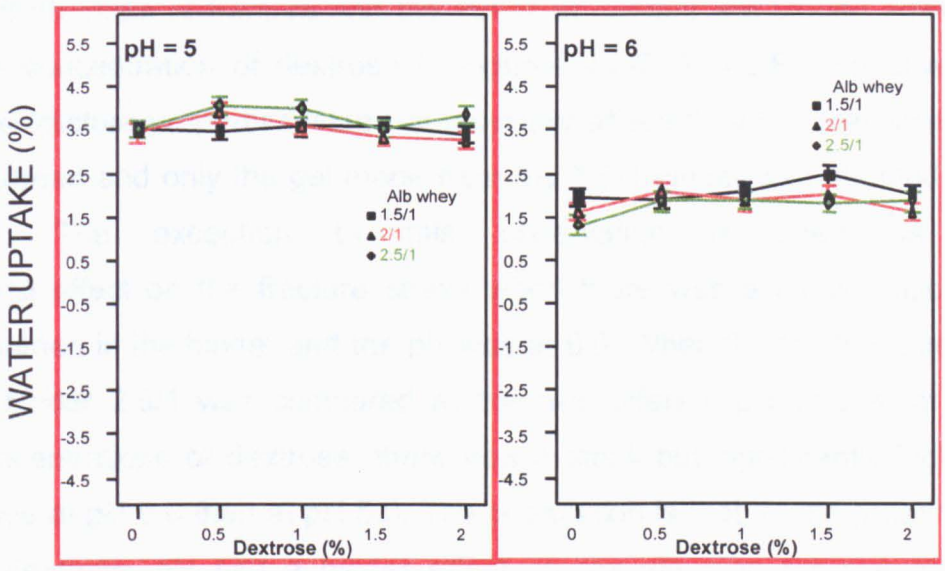
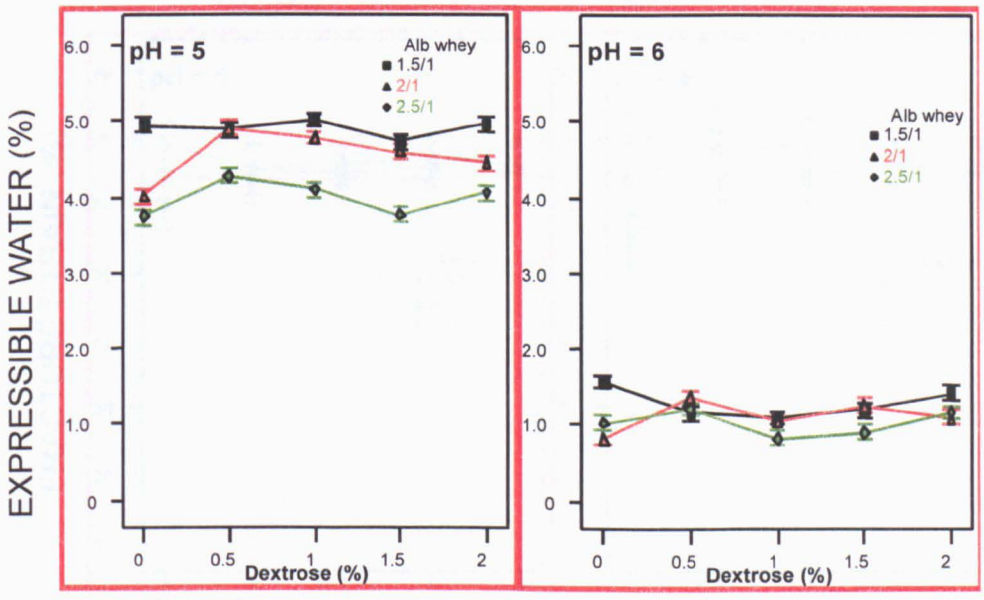


Figure 4.2-D

Effect of Dextrose on Expressible Water



The expressible water, as a measure of the water holding capacity, increases if the spatial structure of the gels makes it easier for water to flow out of the gel. Large pores inhibit the ability of the gel to immobilize water by way of capillary forces (Verheul & Roefs, 1998; Ikeda & Foegeding, 1999).

Fracture Properties

Both strain at fracture and the stress at fracture are plotted against the concentration of dextrose in Figures 4.2-E & 4.2-F respectively. The fracture properties were not generally affected by the presence of dextrose and only the gel made from the 1.5/1 binder was affected by pH. The exception to this observation is seen as a small effect on the fracture stress when there was a higher ratio of albumen in the binder and the pH was at 6.0. When the fracture stress of binder 2.5/1 was compared at the two different pH values, at all concentrations of dextrose, there was a small but significantly higher value at pH 6.0 than at pH 5.0. The postulation is that, in the presence of dextrose, pH had a bigger effect on the albumen (or one of its components) than it did on the whey protein concentrate (or one of its components).

Figure 4.2-E

Effect of Dextrose on Fracture Strain

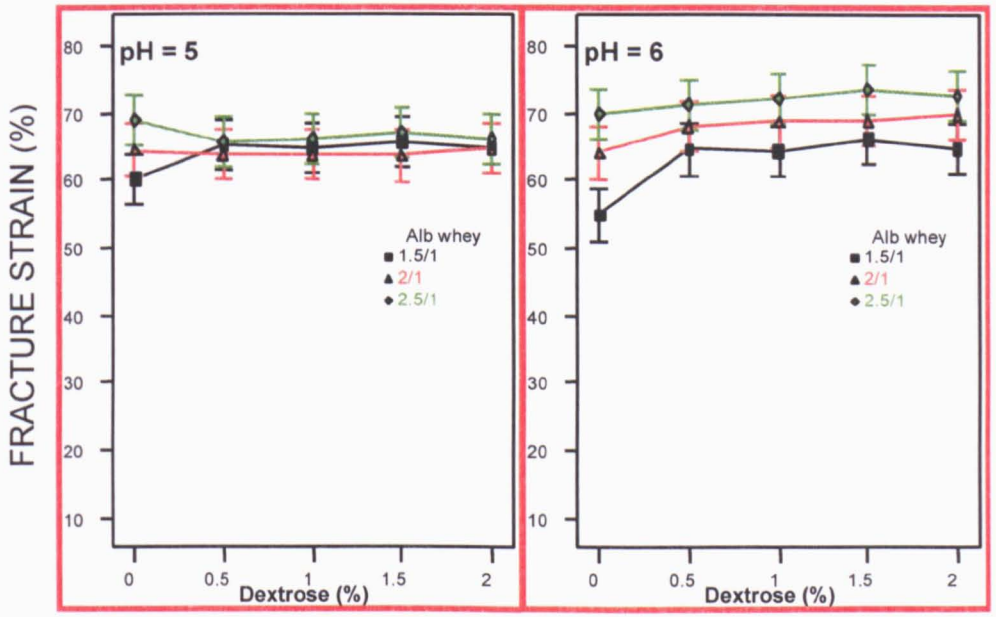
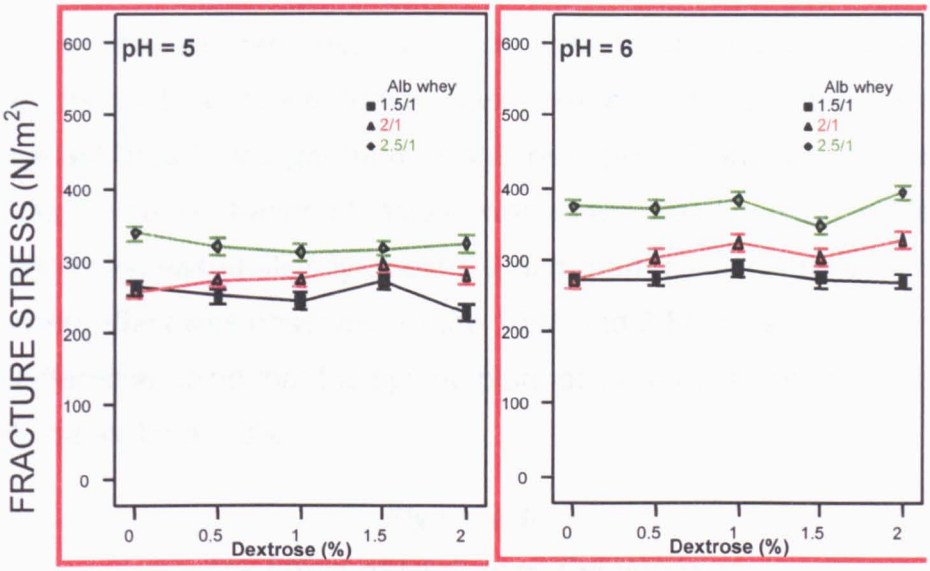


Figure 4.2--F

Effect of Dextrose on Fracture Stress



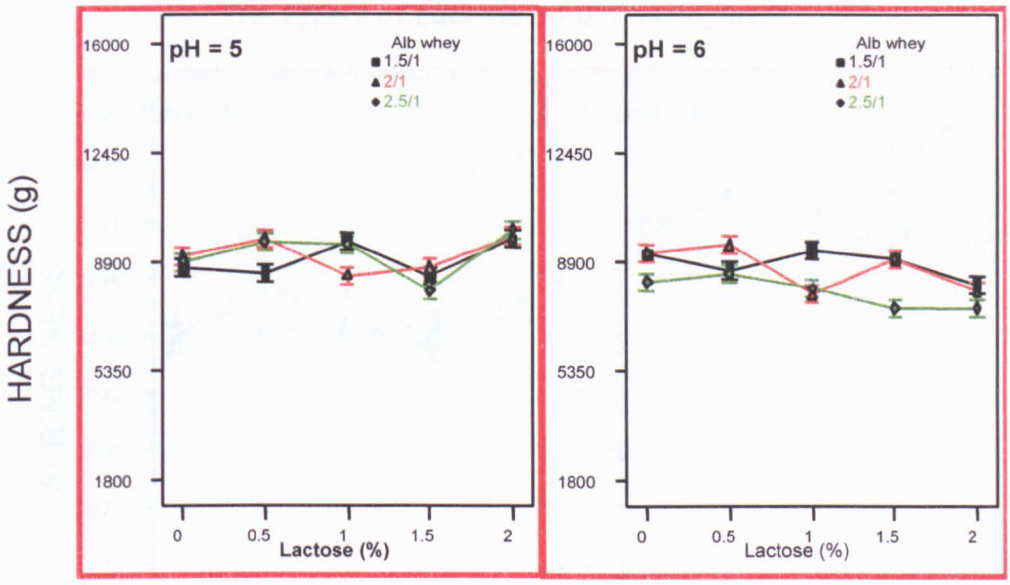
4.3 The Effect Of Lactose

Elastic Properties

At both pH values tested, lactose did not have a consistently lasting effect on the gel hardness at concentrations up to 2% (Fig 4.3-A). When the lactose concentration was in excess of 0.5%, there was a significant drop in the gel hardness at both pH 5.0 and 6.0 for the 2/1 gel. As the concentration of lactose was increased above 1.0%, the gel hardness increased slightly at pH 5.0 and more significantly at pH 6.0. A similar effect was observed for the 1.5/1 and 2.5/1 gels at pH 5.0 with the difference being that the tipping point occurred at a concentration of 1.5% rather than 1.0%.

Figure 4.3-A

The Effect of Lactose on Gel Hardness



It suffices to note that at 2.0% lactose concentration there was no difference between the hardness of gel samples made from any of the three binders at each pH value. The gel formed at pH 5.0 (lactose concentration 2.0%) was significantly harder than the equivalent sample at pH 6.0. In section 3.1.1, it was evident that when pH was the only variable, the higher the pH value between 5-8, the harder the gels. Lactose must therefore have had an ameliorating effect on gel hardness by interfering with the formation of a harder gel at a higher

pH value. In fact at a concentration of 2%, the gels formed at pH 5.0 were harder than the gels formed at pH 6.0.

Plastic Properties

The springiness for the gel samples is shown as a function of the lactose concentration in Figure 4.3-B. The springiness was higher at pH 6.0 than at pH 5.0 irrespective of the amount of added lactose. The *a*-value showed no change with the addition of the lactose as shown in Fig 4.3-G. The implication of this observation is that the viscous properties of the binder systems stayed the same in the presence of lactose.

Figure 4.3-B

The Effect of Lactose on Gel Springiness

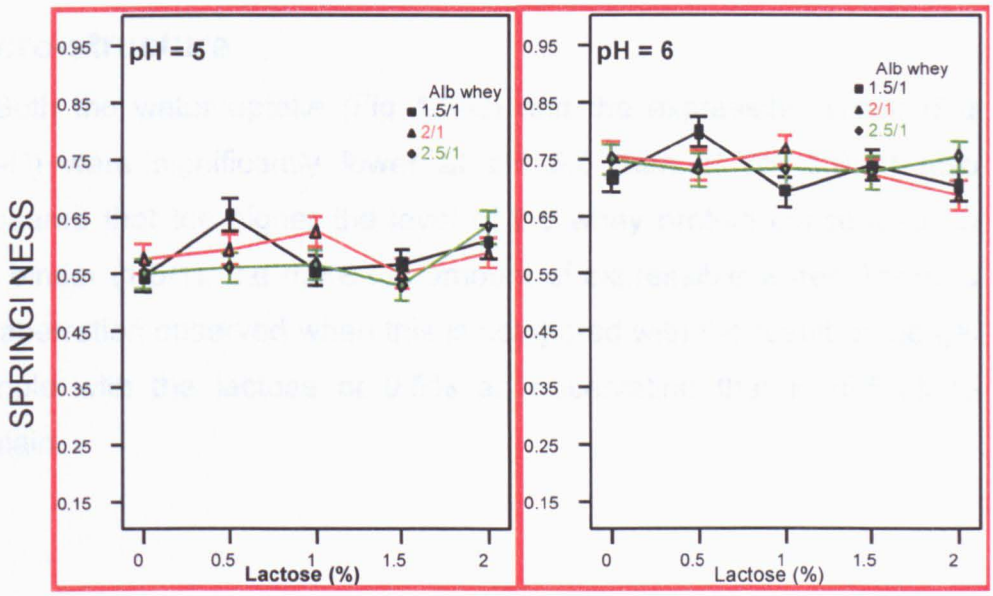
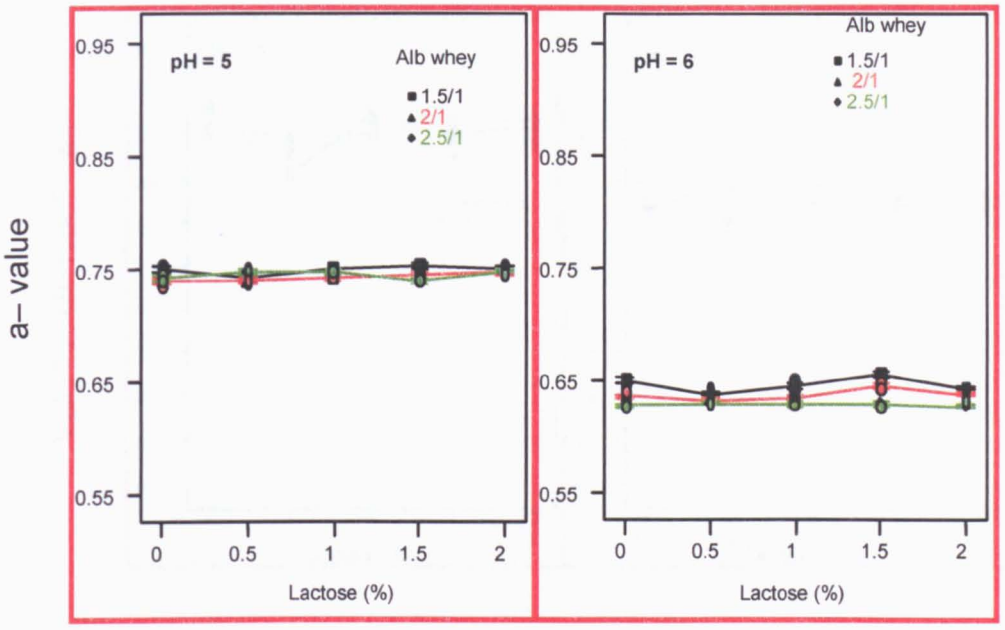


Figure 4.3-G

The Effect of Lactose on Residual Stress



Micro-structure

Both the water uptake (Fig 4.3-C) and the expressible water (Fig 4.3-D) were significantly lower at pH 6.0 than at pH 5.0. It also appeared that the higher the level of the whey protein concentrate in the binder (1.5/1), the more the amount of expressible water. There is an aberration observed when this is compared with the result of the gel sample with the lactose at 0.5% an observation that is difficult to explain.

Figure 4.3-C

The Effect of Lactose on Water Uptake

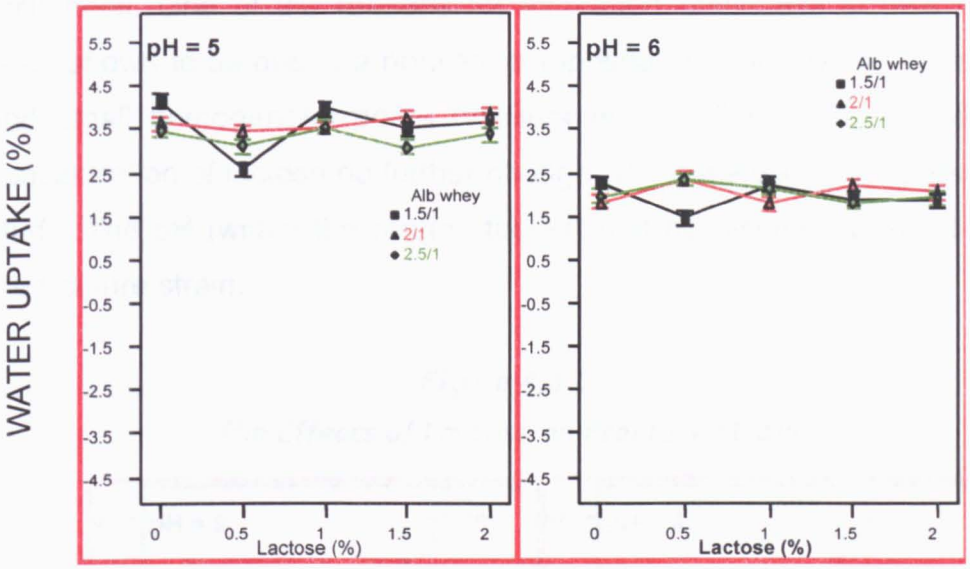
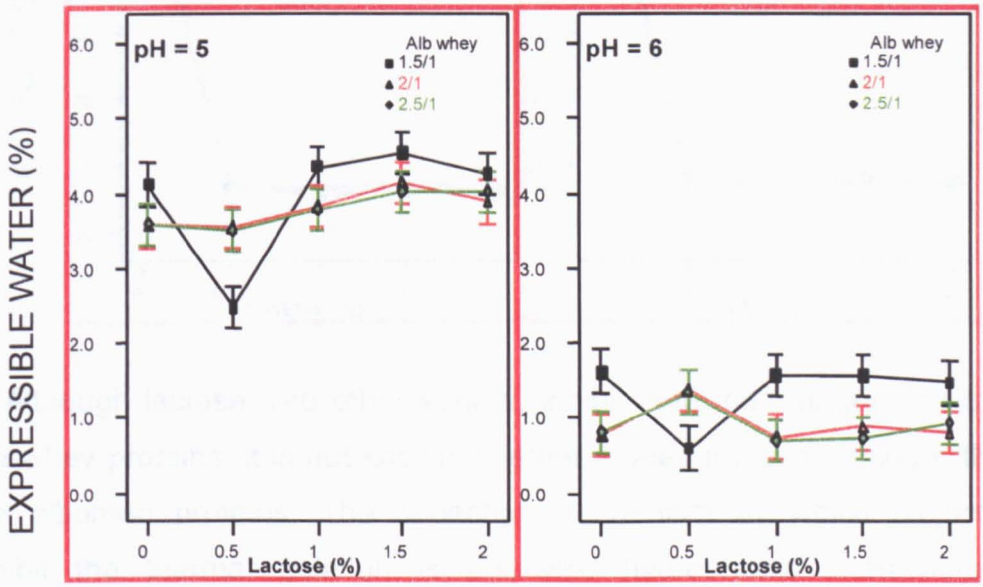


Figure 4.3-D

The Effects of Lactose on Expressible Water

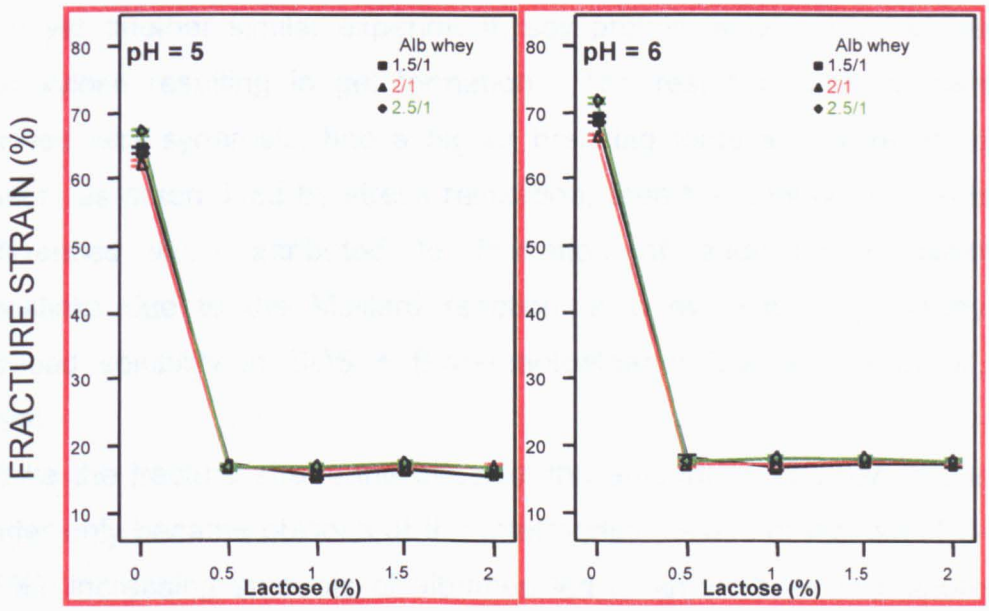


Fracture Properties

Fracture strain (Fig 4.3-E) falls rapidly in the presence of as little as 0.5% lactose. Reduced fracture strain is indicative of increased brittleness. One of the reasons for increased brittleness in gels has been shown to be due to a network composed of relatively thin strands and small homogenous pores (Weijers *et al.,* 2006). At increased concentration of lactose no further change was observed in the present study. The pH (within the range studied) had no discernible effect on the fracture strain.

Figure 4.3-E

The Effects of Lactose on Fracture Strain



Although lactose, like other sugars, inhibits the thermal gelation of the whey proteins, it is not known whether it does the same thing with the albumen proteins. The accepted mechanism by which sugars inhibit the thermal gelation is pair-wise hydrophobic interactions involving both the solvent and the protein (Garrett *et al.*, 1988). Tang *et al.* (1994) proposed an alternative mechanism by which lactose inhibits whey protein gelation through the formation of Schiff base products between lactose and the amine groups of the whey protein.

There could be another reason for the change in fracture strain in the presence of lactose. Hill *et al.,* (1992) reported that when xylose

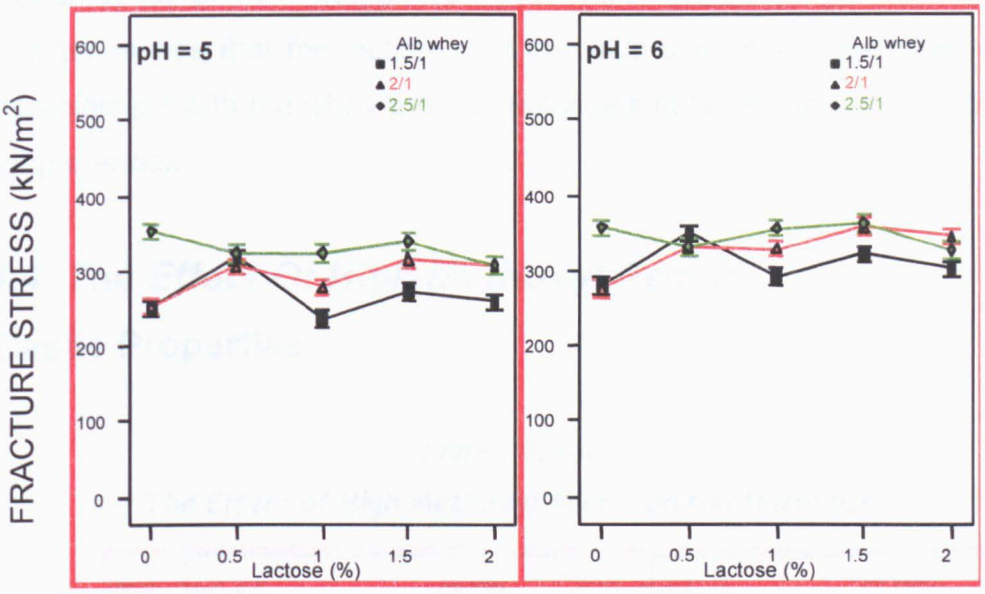
was heated in the presence of Bovine Serum Albumen (BSA), there was, amongst other observations, the formation of additional covalent bonds as evinced by the insolubility of the so-called Maillard gels in a solution of SDS + β-mercaptoethanol. A further experiment identified that aggregates formed in a mixture of xylose and BSA heated at 95°C for up to 80 minutes involved non-disulphide covalent linkages resulting from the Maillard reaction (Easa *et al.*, 1996). In this study, a plot of the diffusion coefficient against molar mass suggested that the Maillard gels aggregates grew in a linear, 'string of beads', fashion most likely resulting in a fine-stranded gel. Fine stranded Maillard gels are more likely to be brittle and exhibit low strain at fracture (Mitchell and Hill, 1995; Weijers *et al.*, 2006).

In yet another similar experiment, soy protein isolate was heated with xylose resulting in gel formation. The resulting Maillard gels showed less syneresis, had a higher breaking force and were more elastic, as determined by stress relaxation, then the control gels. The differences were attributed to formation of additional covalent crosslinks due to the Maillard reaction, also evidenced by greatly reduced solubility in SDS + β-mercaptoethanol (Cabodevila *et al.*, 1994).

Like the fracture strain, the effect of the amount of albumen in the binder only became obvious at the intermediate levels of lactose (1.0-1.5%): increasing amounts of albumen led to an increase in fracture stress (Fig.4.3-F).

Figure 4.3-F

The Effects of Lactose on Fracture Stress



Schmidt *et al.* (1978) reported that dialysis of whey protein concentrate in order to reduce the protein/lactose ratio from 2:1 to 100:1 produced a gel of considerably increased strength.

Most sugars tend to increase the gelation time and reduce the rate of gelation. The effect is thought to be due to the ability of these sugars to increase the surface free energy between water and a hydrophobic surface (Kulmyrzaev *et al.*, 2000). Bryant & McClements (2000b) suggested that this phenomenon occurs because sucrose increased the viscosity of the continuous phase and therefore slowed the movement of the aggregates. However they also observed that at high levels of sucrose (>6%), the gel time decreased, likely due to the increased efficiency of the protein-protein collision enhancing protein molecule aggregation.

Schmidt *et al.* (1979) also observed that gels formed from dialysed whey protein concentrate (i.e. with the removal of lowest molecular weight components such as lactose) were more translucent, stronger, gummier and chewier than gels formed from non-dialysed whey protein concentrate.

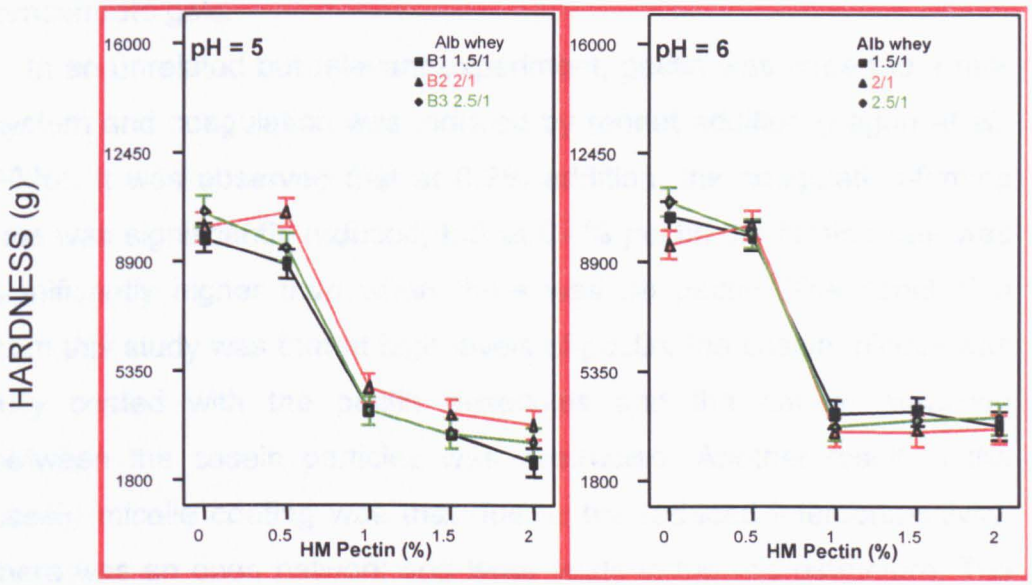
Haggett (1976) showed that there is an interaction between pH and salt content. In a series of experiments Haggett (1976) proved that whey protein prepared from acid whey by dia-filtration had good gelling properties at pH 6.0 but poorer gels were formed at pH 8.5. The conclusion was that the lactose and the salts somehow collaborate at the acidic pH with the whey protein molecules to beneficially modify the gel properties.

4.4 The Effect Of High Methoxyl Pectin

Elastic Properties

Figure 4.4-A

The Effect of High Methoxyl Pectin on Gel Hardness



As shown in Figure 4.4-A, the rising concentration of pectin led to a steep drop in gel hardness for all binder samples. There was an initial range (<0.5%) in which there was no response. At both pH values, the effect of the pectin, in affecting gel hardness appeared to have been completed by the time the concentration reached 1.0%. Outside this region, the gel hardness became relatively insensitive to increased concentration of pectin.

A test carried out by Beaulieu *et al.* (2001) showed that increasing the degree of methoxylation in low methoxyl pectin had the effect of increasing the protein aggregation of a whey protein gel. Due to the fact that increased aggregation produces a particulate gel, it is perhaps no surprise that in Figure 4.4-A, the escalating concentration of pectin led to an increasingly softer gel.

Another possible explanation may be that because of the affinity of pectin for water, the solvent may be preferentially partitioned between the protein and the hydrocolloid during the gelation process. Many pectin products gel when cold, therefore a higher molar concentration of the water may be accounted for in the pectin phase. Beaulieu *et al.* (2001) also reported that mixed gels of whey protein concentrate and pectin retain more water and were less brittle than pure whey protein concentrate gels.

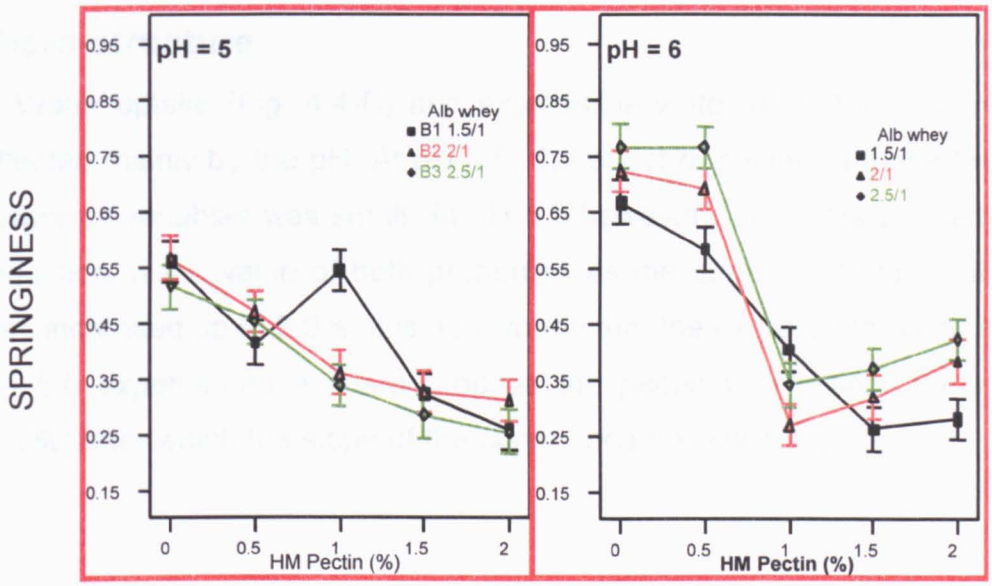
In an unrelated but relevant experiment, pectin was added to a milk system and coagulation was induced by rennet addition (Fagan *et al.*, 2006). It was observed that at 0.2% addition, the coagulation-firming rate was significantly reduced, but at 0.4% pectin the firming rate was significantly higher than when there was no pectin. The conclusion from this study was that at high levels of pectin, the casein micelle was fully coated with the pectin molecules and the natural attraction between the casein particles was obstructed. Another result of the casein micelle coating was that due to the reduced interconnectivity, there was an open network and large voids in the microstructure. The researchers concluded that the initial decrease in the coagulation-firming rate might have been due to the increased viscosity of the milk caused by the addition of pectin (Fagan *et al.*, 2006). In the present study, a similar increase in viscosity was visually observed with the addition of pectin to the binder system prior to the heat-induced gelation. However, it is unknown whether a similar mechanism is taking place whereby the pectin molecules similarly coat the whey protein concentrate and/or albumen molecules.

Plastic Properties

Gel springiness was steadily reduced by the addition of the pectin to the binder at pH 5.0 (Fig 4.4-B). At pH 6.0, there was a lag in reaction of the springiness to an increase in the concentration of the pectin, particularly with the samples 2/1 and 2.5/1. The reduction in value did not commence until a concentration of 0.5% had been achieved and appeared to have been completed by the time the pectin concentration reached 1.0%. After this point, additional pectin had no further effect on the springiness.

Figure 4.4-B

The Effect of High Methoxyl Pectin on Gel Springiness

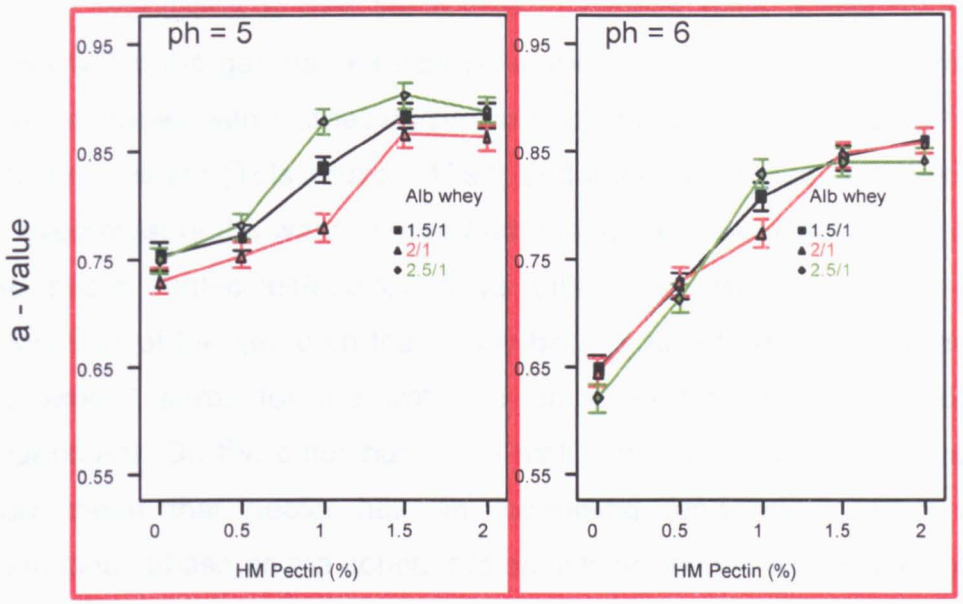


The change in the *a*-value as shown in Fig. 4.4-G varied inversely with the reduction in springiness. The *a*-value showed a significant increase as the amount of pectin was raised. The increase in the *a*-value showed that the gel had become more viscous and less elastic.

A higher *a*-value is also indicative of weak molecular interactions (Messens *et al.*, 2000).

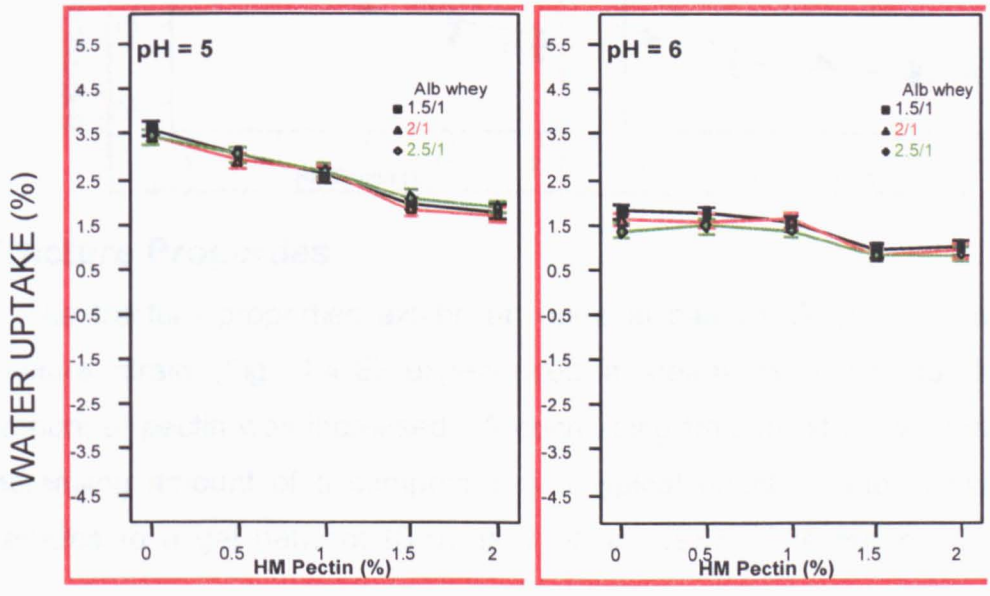
Figure 4.4-G

The Effect of High Methoxyl Pectin on a - Value



Micro-structure

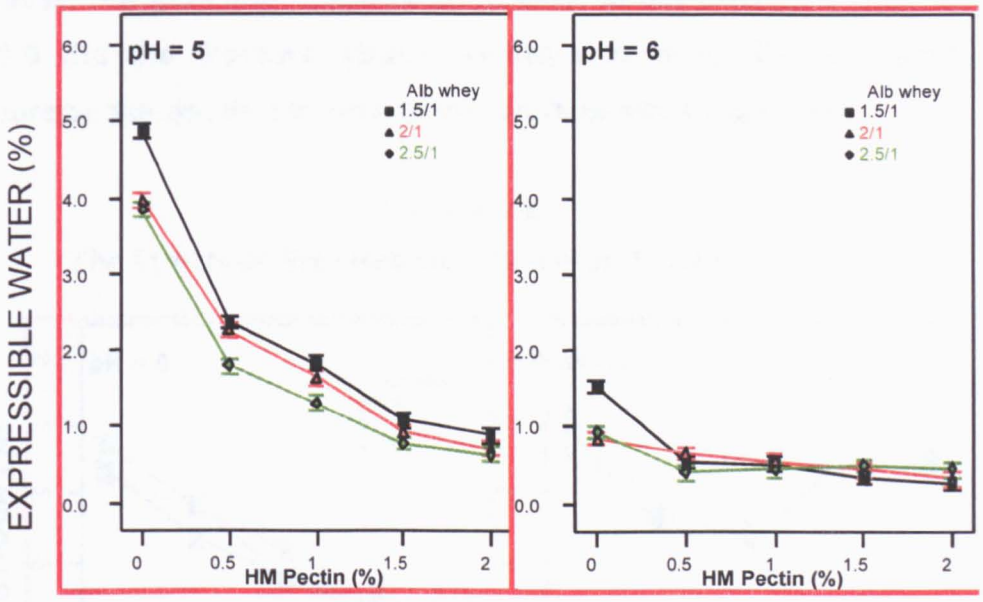
Water uptake (Fig. 4.4-C) and expressible water (Fig. 4.4-D) were affected mainly by the pH. At pH 6.0, the effect of pectin on these two response variables was small. At pH 5.0 however, there was a steady reduction in the value of both properties as the quantity of the pectin was increased up to 2.0%. It is noteworthy that the expressible water at pH 5.0 experienced a sharp drop as the pectin was initially added (0.5%) after which the slope of the curve became less steep.



The hypothesis is that the pectin molecules were acting as inert fillers within the gel matrix because of the fact that the residual stress was increased with increasing pectin concentration. Pectin has a high affinity for water (Tolstoguzov, 1991) and if the pectin has absorbed all or even most of the water before and during the steaming process or if the pectin molecules/strands have taken up residence within the interstices of the gel, then there may be a reduced amount of or even no 'empty' pores for the water to go to during the water uptake experiment. On the other hand, the high attraction of pectin for water may mean that pectin has an increasing tendency to form the continuous phase as the concentration is increased (DeMars & Ziegler, 2001). Under these circumstances, it may well be that a pectin-continuous gel was formed when the pectin was in excess of 0.5% with the protein gel forming a 'discrete' phase.

Figure 4.4-D

The Effects of High Methoxyl Pectin on Expressible Water



Fracture Properties

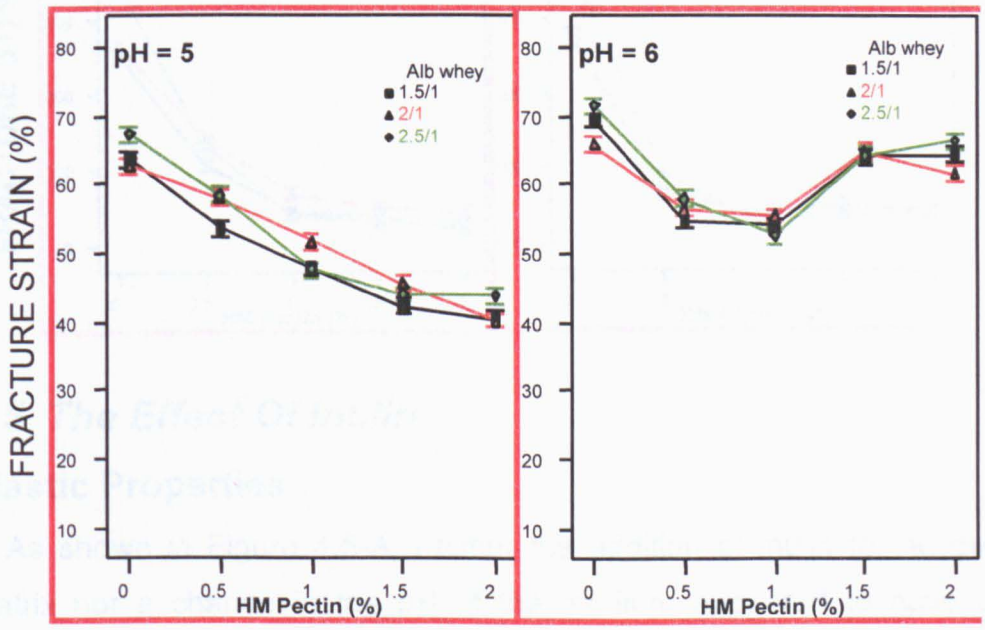
The fracture properties exhibit an unusual pattern. At pH 5.0, the fracture strain (Fig. 4.4-E) experienced a steady reduction as the amount of pectin was increased. A decreasing fracture strain with the increasing amount of a compound is a typical effect of adding filler particles to a gel network (Brownsey *et al.*, 1987). The reduction in

fracture strain showed that the gel was more brittle and this is to be expected, especially if there was no interaction between the added ingredient and the gel network (Tavares & Lopes da Silva, 2003).

When there is no interaction between the filler material and the gel network, the fracture strain is much less dependent on the volume fraction or the size of the filler particles (Ikeda & Foegeding, 1999). In the present study, however, at pH 6.0, there is a nadir at about 1.0 % pectin after which a shift occurred and the fracture strain started to increase. The likely explanation is that as pectin was added to the binder, the pectin molecules acted as inactive filler particles, in which the pectin gel was interspersed within the whey protein concentrate/albumen gel leading to increased brittleness. At the tipping point of 1.0%, there was an inversion, whereby the whey protein/albumen gel became the inactive filler in a pectin gel (Ikeda & Foegedding, 1999) or part of an inter-penetrating or phase separated gel (Boye *et al.*, 2000). As such, the overall brittleness was reduced. **However, the question remains as to why, in the present study, at pH 5.0 did the fracture strain continue to drop. Did the acid encourage the pectin strands to continue to stick together?**

Figure 4.4-E

The Effects of High Methoxyl Pectin on Fracture Strain

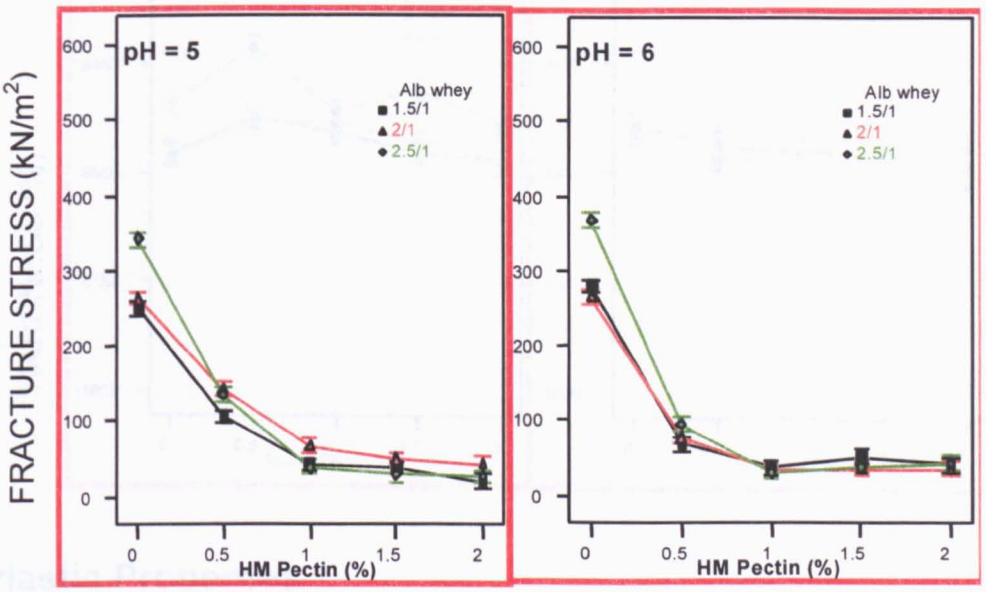


The pH did not have an effect on the fracture stress (Fig. 4.4-F). In addition, the amount of pectin did not have any further effect once the concentration was in excess of 1.0% as shown by the 'flat-lining' in Fig.4.4-F. The reduction in fracture stress with increasing pectin (0-1%) suggests that the pectin is almost certainly acting as an inert filler. Inert fillers are known to cause a reduction in stress (Lucey *et al.*, 1999). Other likely scenarios were that either an inter-penetrating pectin gel was produced or there was complete phase separation in which there was a pectin gel dispersed into a whey protein/albumen continuous gel or vice versa.

The reduction in fracture strain or increase in brittleness with increasing amount of pectin may also be due to the fact that the strands in the network have thicker strands and large homogenous pores (Weijers *et al.*, 2006).

Figure 4.4-F

The Effect of High Methoxyl Pectin on Fracture Stress



4.5 The Effect Of Inulin

Elastic Properties

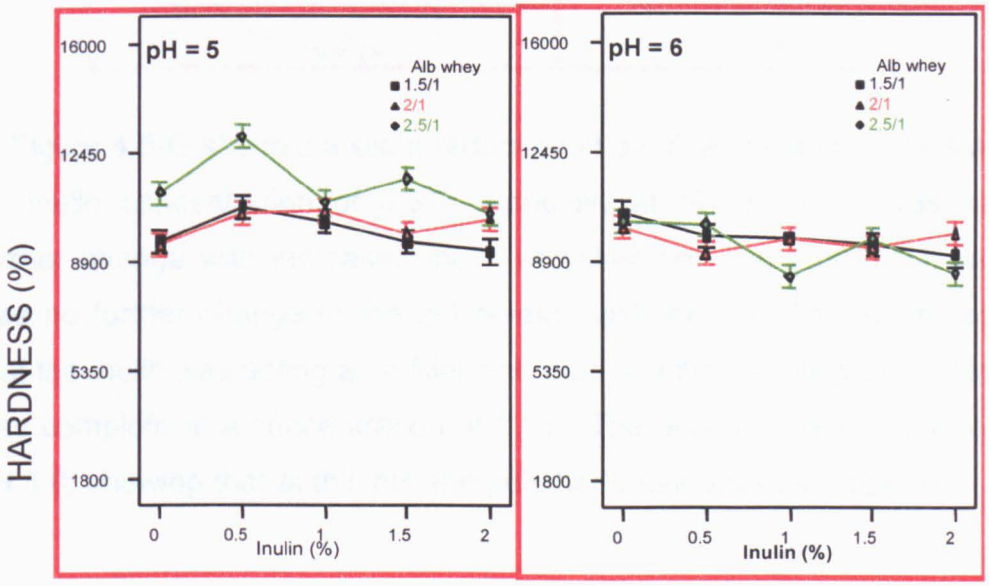
As shown in Figure 4.5-A, neither the addition of inulin to the gel matrix nor a change in the pH of the medium appeared to have a

coherent effect on the gel hardness. At pH 5.0, there was a small but significant increase in the gel hardness for all three gel samples but only as the inulin concentration was raised from 0 - 0.5%, especially the 2.5/1 binder. In spite of this, the effect all but slowly dissipated as the concentration of inulin was increased.

In an experiment carried out by Kim *et al.* (2001), it was shown that the effect of pH on the structure of a pure inulin gel (25% w/v) was not different between pH 4–10. In another experiment carried out by Fagan *et al.* (2006), the addition of an increasing amount of inulin (0 – 3%) to a milk system coagulated with rennet produced no change in the gel microstructure. The observation was attributed to the fact that because inulin is relatively short-chained, it was unable to form highly tangled polymer gel systems at low concentrations.

Figure 4.5-A

The Effect of Inulin on Gel Hardness



Plastic Properties

Like the gel hardness, inulin did not show a consistent effect on the springiness although a gel made at pH 6.0 was significantly higher in springiness than one made at pH 5.0 (Fig. 4.5-B). The 1.5/1 gel sample had the lowest value of springiness of all the binders at pH 5.0 but at pH 6.0 there was no difference between the binder samples. The

observation suggests that there was an interaction between the inulin at pH 5.0 and the amount of albumen in the binder. **A pertinent question to ask and which is deserving of further investigations is whether inulin functionality affected by pH?**

Figure 4.5-B

The Effect of Inulin on Gel Springiness

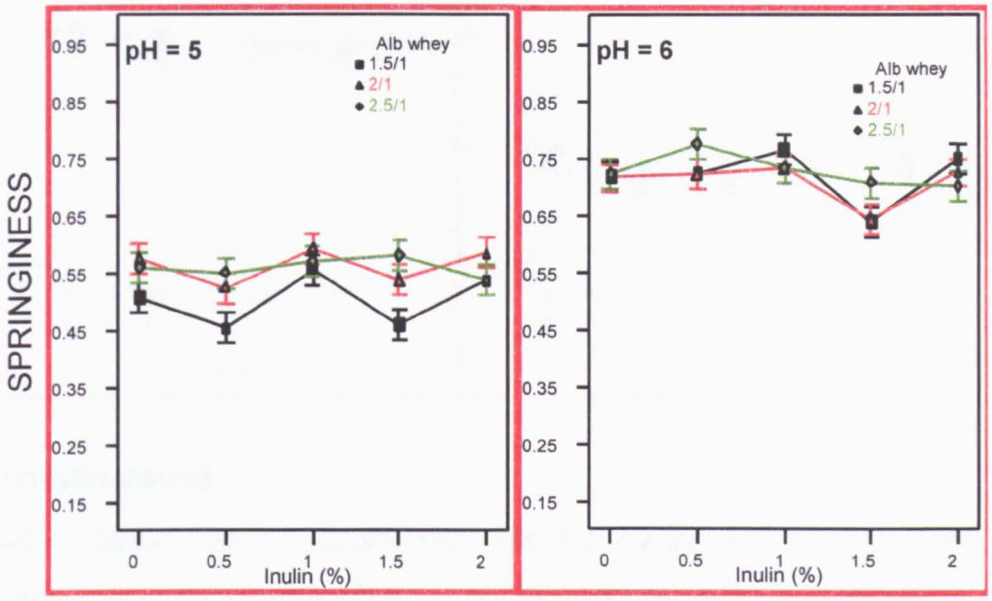
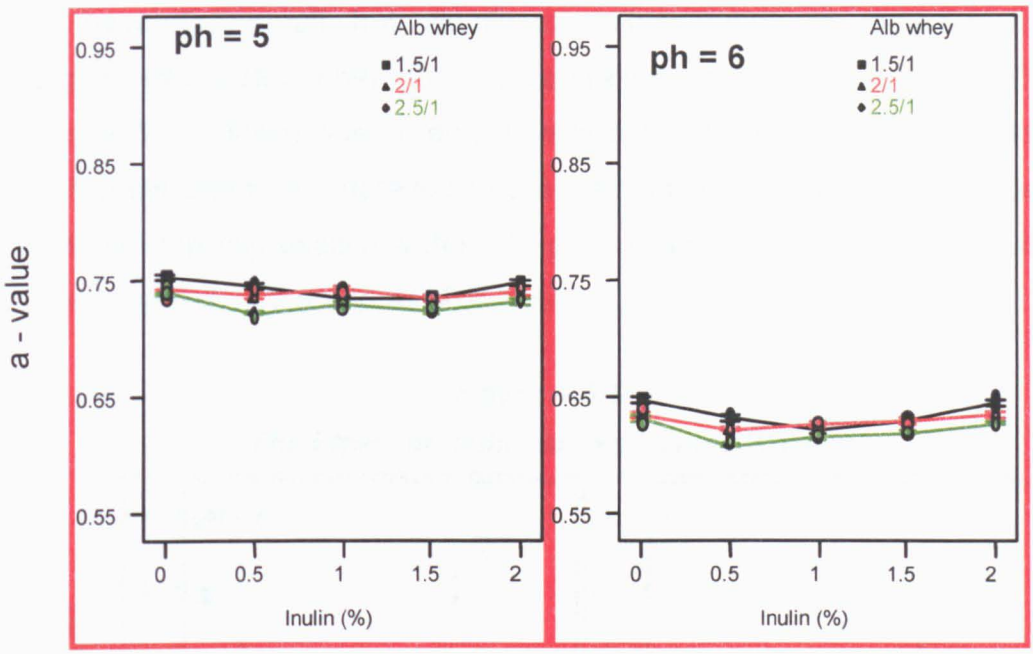


Figure 4.5-G showed a slight reduction in *a*-value in the inulin gels at an inulin concentration of 0.5% especially at pH 6. There was no further change with increased inulin at either pH. The fact that there was no further change in the gel *a*-value with extra inulin, suggested that the inulin was acting as a filler and that its action in filling the pores was complete at a concentration of 0.5%. The *a*-values were higher at pH 5.0, showing that at this pH, the gels had more viscous properties.

Figure 4.5-G

The Effect Inulin on a-Value

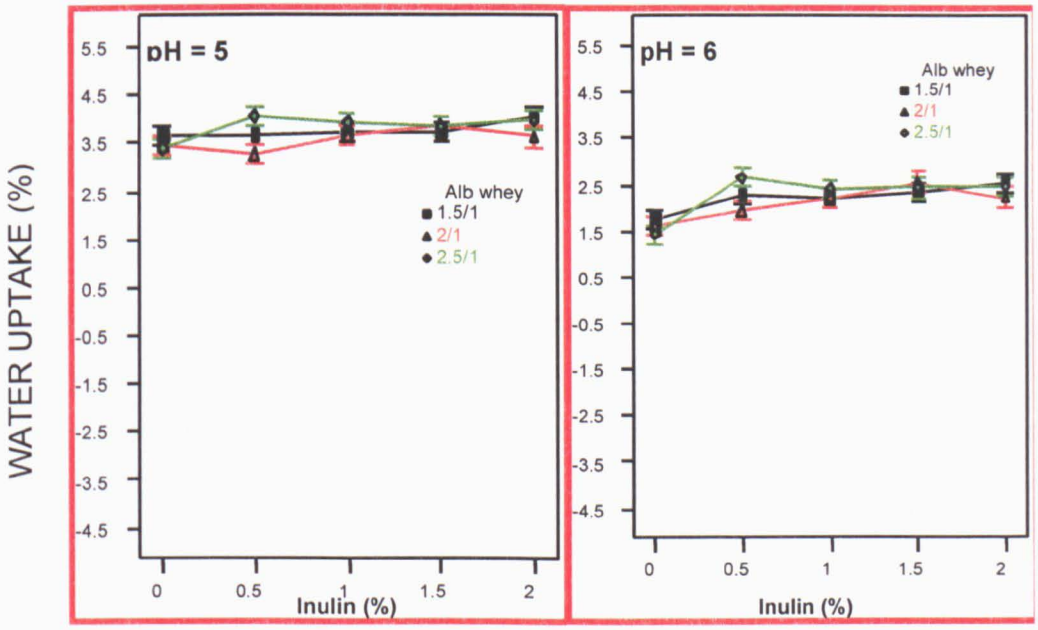


Micro-structure

Water uptake was not significantly affected by the addition of inulin but there was a noticeable effect when the pH was altered. The water uptake was significantly higher at pH 5.0 for all binder types (Fig. 4.5-C) than it was at pH 6.0.

Figure 4.5-C

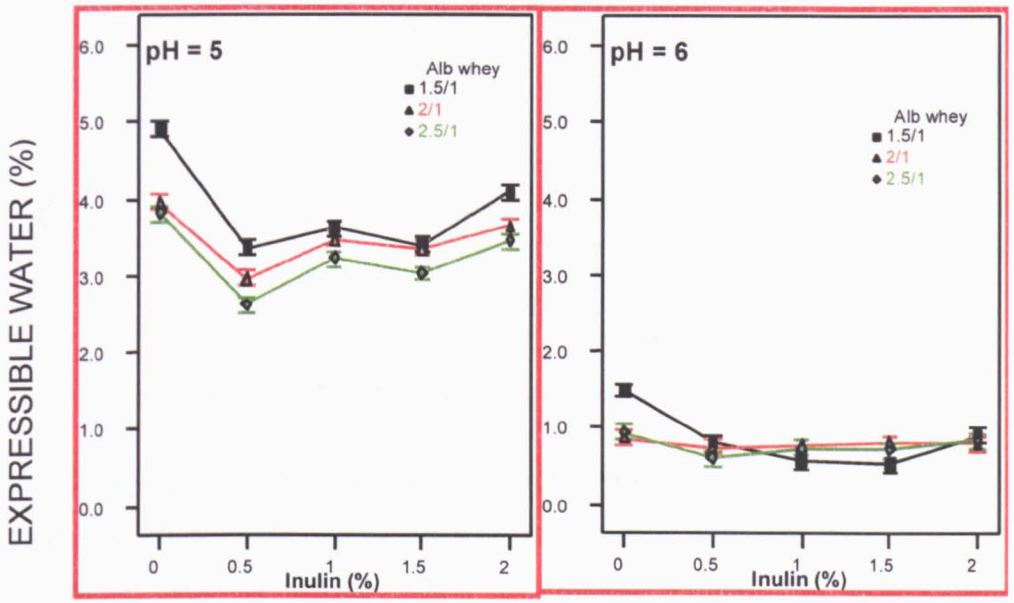
The Effect of Inulin on Water Uptake



Expressible water was also significantly higher at pH 5.0 with the binder samples (Fig. 4.5-D). At this pH value, inulin addition appeared to have the small but significant achievement of reducing the expressible water when the concentration of the inulin was 0.5%. Above 0.5%, there was no evidence of any further change. In addition to the pH effect, an increase in the amount of albumen in the binder reduced the expressible water. At pH 6.0 and above 0.5%, the inulin had no effect on the expressible water.

Figure 4.5-D

The Effect of Inulin on Expressible Water

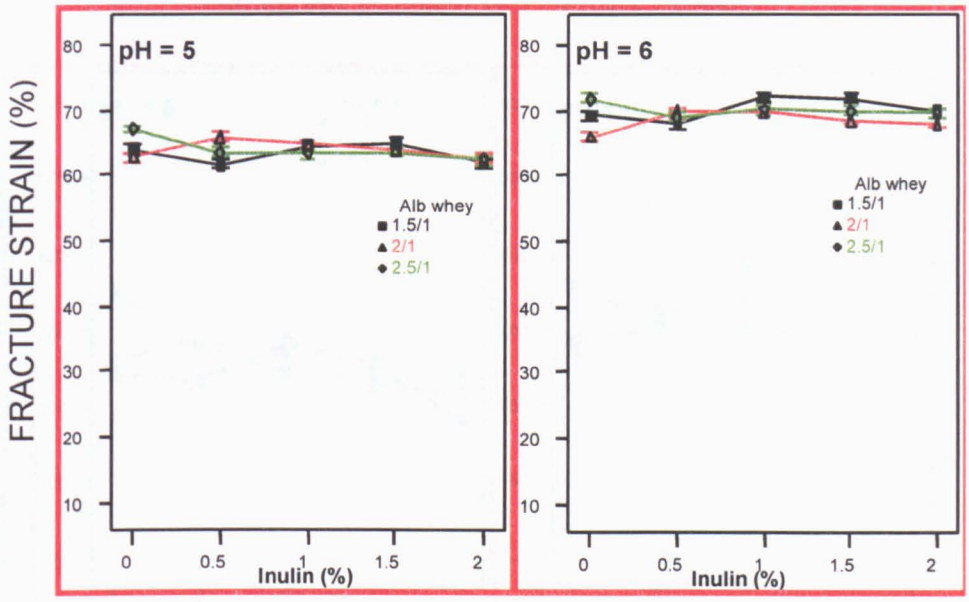


Fracture Properties

Figure 4.5-E shows that the fracture strain was mostly unresponsive to inulin as only very small changes occurred up to 2% concentration.

Figure 4.5-E

The Effect of Inulin on Fracture Strain

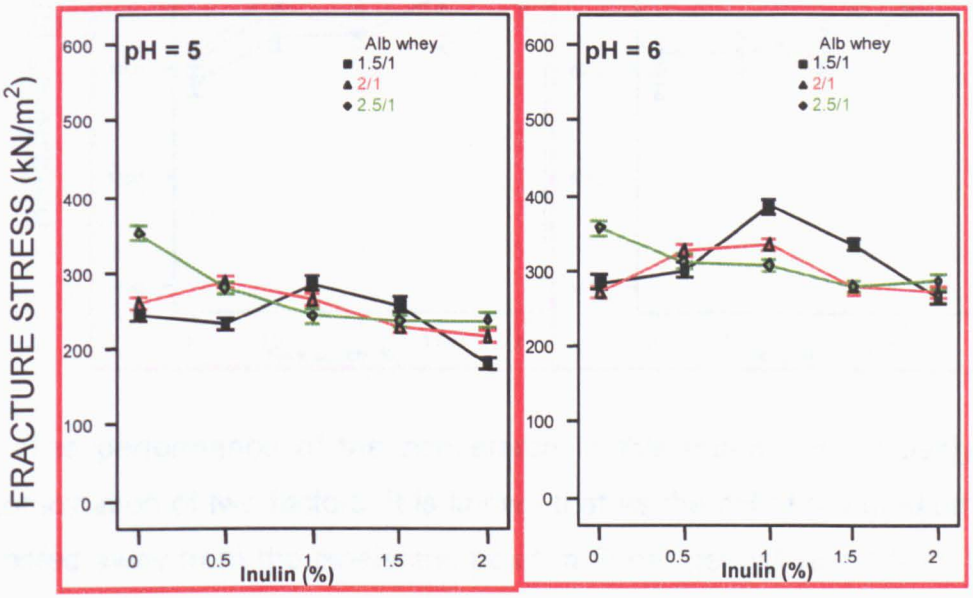


The fracture stress of the 2.5/1 binder sample at pH 5.0 (Fig. 4.5-F) showed a small response to the ever-increasing amounts of inulin up to a concentration of about 1.0%. In reality, it was not significantly different, by and large, to the response shown by the other binder samples. However, the reaction of the 1.5/1 sample to inulin at both pH values, although small, is worthy of note. At 0-0.5% inulin there was no reaction. A peak appeared at both pH values after which addition of further inulin reduced the fracture stress. What can be theorized from binder 1.5/1 in Fig. 4.5-F was that at 0.5% inulin, the whey protein concentrate/albumen gel was not affected by the inulin. As the inulin was increased further, it likely acted on the protein gel in one of three ways (1) by binding the water, (thus concentrating the dry protein) or (2) by acting as filler or (3) by forming a gel in competition with the protein. Such a gel is likely to be a phase-separated gel due to the inability of inulin to form a true gel (Hennelly *et al.*, 2006).

In addition, a pure inulin gel is known to exhibit increasing fracture stress as the concentration is increased (Bot *et al.*, 2003). Therefore, it is quite possible that the reason why there is reduced fracture stress with concentration of inulin in excess of 1.0% in the 1.5/1 gel is due to the increasing influence of an inulin-rich phase in the gel matrix.

Figure 4.5-F

The Effect of Inulin on Fracture Stress



4.6 The Effects Of Rice Starch

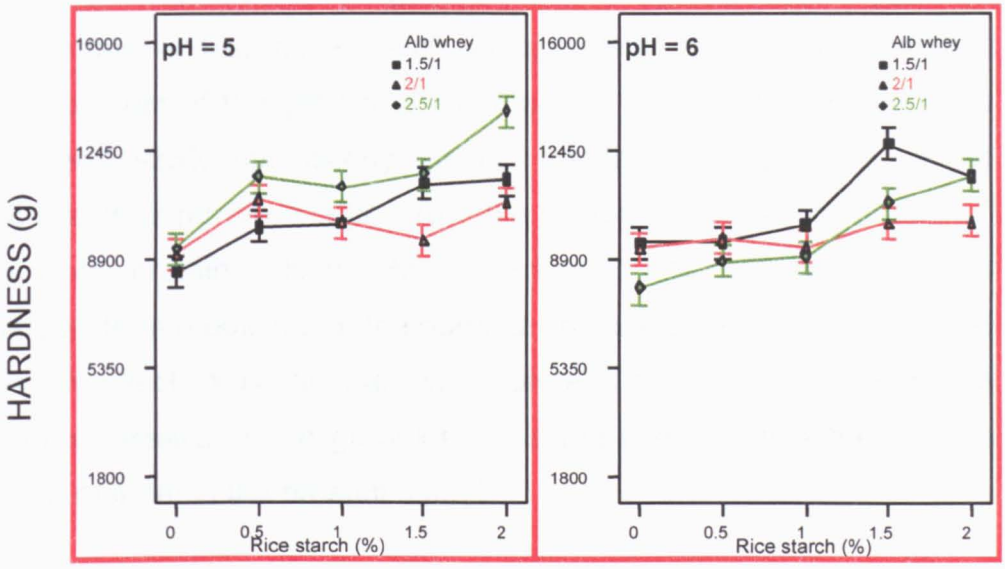
Elastic Properties

In Figure 4.6-A, when the concentration of rice starch is plotted against the resulting gel hardness, there is evidence that rice starch increases the gel hardness. At pH 5.0, the increase in gel hardness was observable immediately as soon as the concentration was raised from 0% to 0.5%. All the binders showed increased gel hardness. At the intermediate concentration (0.5-1.5%), there was a lag before another step change at 2.0% rice starch. At pH 6.0, no change was noted until the concentration of rice starch had reached about 1.5% for all three binder systems. There is an interaction between the rice starch, the pH and the binder type. As the proportion of albumen in the gel was increased, the hardness increased at pH 5.0 as evinced by the

2.5/1 binder being the hardest. A shift then occurs when the pH was changed such that the 1.5/1gel is the hardest at pH 6.0.

Figure 4.6-A

The Effect of Rice Starch on Gel Hardness



The performance of the rice starch in this manner is probably a combination of two factors. It is known that as the pH of the medium is shifted away from the isoelectric point, a firmer gel will result from the proteins because of the more favourable conditions for the sulphydryl to disulphide reaction. However at pH 5 in the present study, the acidic conditions probably affected the rice starch as well as the whey protein. Wang *et al.*, (2000) showed a clear effect of pH on rice starch viscosity. The researchers demonstrated that a rice flour paste displayed a lower viscosity profile at pH 4.10 than at pH 6.20. It was attributed to the fact that at pH 4.10, the rice starch granules, in the presence of heat, became fragile and broke down very quickly after acidification. The starch paste also showed less tendency of retrogradation and was ascribed to the short chain starch molecules that were too active to form an ordered crystalline structure. The increase in gel hardness at the higher pH may also indicate that the starch was behaving as active filler (Sok Line *et al.*, 2005).

In an experiment carried out by Lee & Rhee (2007), it was shown that increasing concentration of rice starch leads to an increase in gel

hardness up to about 3% when the heating temperature was 90°C. A lower pH favours the production of an opaque, particulate gel. The hypothesis presented in this study may have been corroborated by another study carried out with cassava starch. In a series of experiments carried out by Aguilera & Baffico (1997) with whey protein concentrate and cassava starch, compression testing confirmed that reinforcement of the gel occurred when the starch concentration was between 10-25%. Microscopy revealed that the starch granules swelled first and thus removed water from the system, thereby 'concentrating' the whey protein solution that gelled later into one with a higher modulus than predicted for the nominal concentration. The resulting gel was, in effect, a continuous whey protein gel filled with particulate swollen cassava starch granules. Was the rice starch behaving in a similar manner in the present study?

Plastic Properties

Increasing the concentration of the rice starch did not have much effect on the springiness of the gel at pH 5.0. At pH 6.0, there was a steady increase observed for the gel springiness until the concentration reached about 1.0%, after which there was no further change (Fig. 4.7-B). The observation is loosely in agreement with the work of Lee & Rhee (2007), although pH was not used as a variable in their study. They noted an increase in springiness up to 3% rice starch concentration, above which there was no further increase in the springiness.

Figure 4.7-G shows a steady reduction in the *a*-value as the rice starch is increased at pH 5.0. The reduction is tantamount to an increase in the intermolecular interactions, corresponding to the increase in the gel hardness observed in Fig 4.7-A. At pH 6.0, there was only a limited change in the *a*-value at fracture between rice starch concentration of 0 – 0.5%.

Figure 4.6-B

The Effect of Rice Starch on Gel Springiness

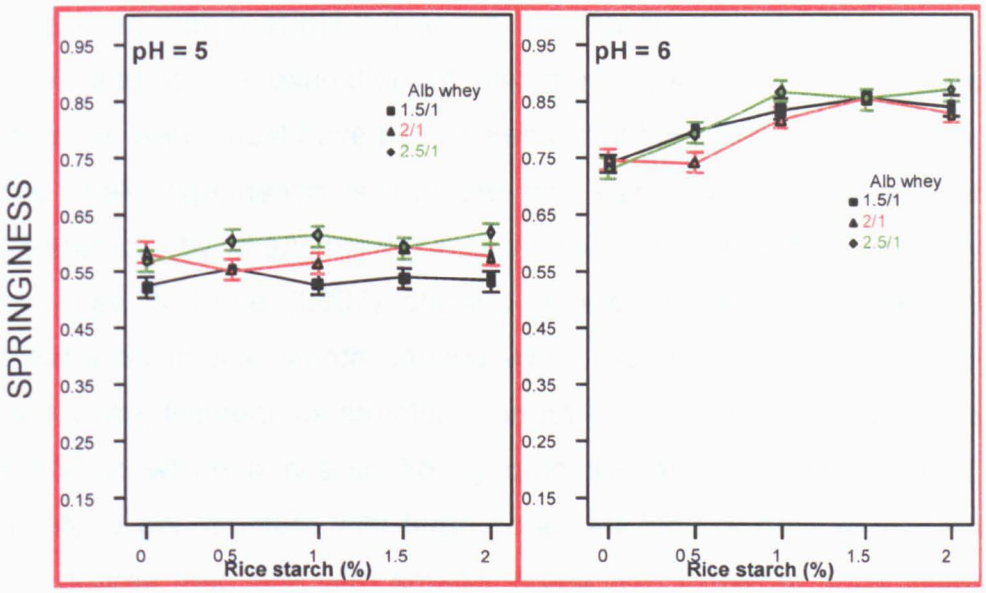
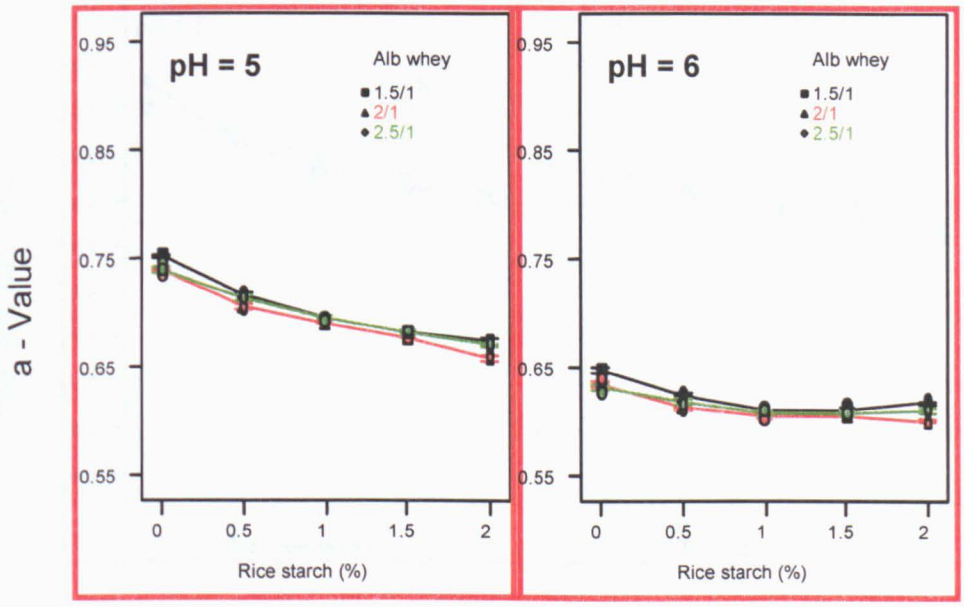


Figure 4.6-G

The Effect Rice Starch on a - Value



Micro-structure

Both the water uptake and the expressible water were progressively reduced as the amount of rice starch was increased (Figs. 4.6-C & 4.6-

D). The rate of reduction was slightly more pronounced at pH 5.0 than 6.0 for both response variables as shown by the steeper slope at pH 5.0. Expressible water was affected at all rice starch levels at pH 5.0. Adding extra starch at pH 6.0 above a concentration of 0.5% starch, did not lead to the exudation of any more water: all the available expressible water must have been taken out at the lower concentration.

The likely explanation is that the rice starch granules affect the microstructure by changing the size of the pores and their capillary action. Lee & Rhee (2007) showed in their experiments that 1% concentration of rice starch caused whey protein concentrate gel to have a loose, filamentous structure compared to those prepared at 3% and 5%, in which a coarse honeycomb like structure was formed. Normally, a gel structure with large pores will bind or hold water to a lesser degree than one with smaller pores and therefore such water can be expelled more easily under pressure (Hermansson, 1986).

Figure 4.6-C

The Effect of Rice Starch on Water Uptake

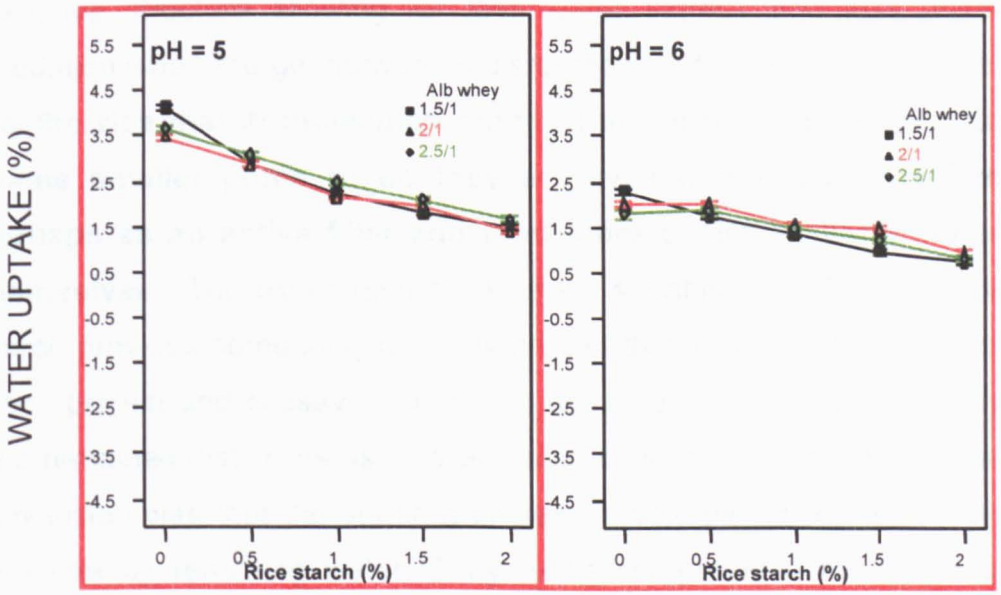
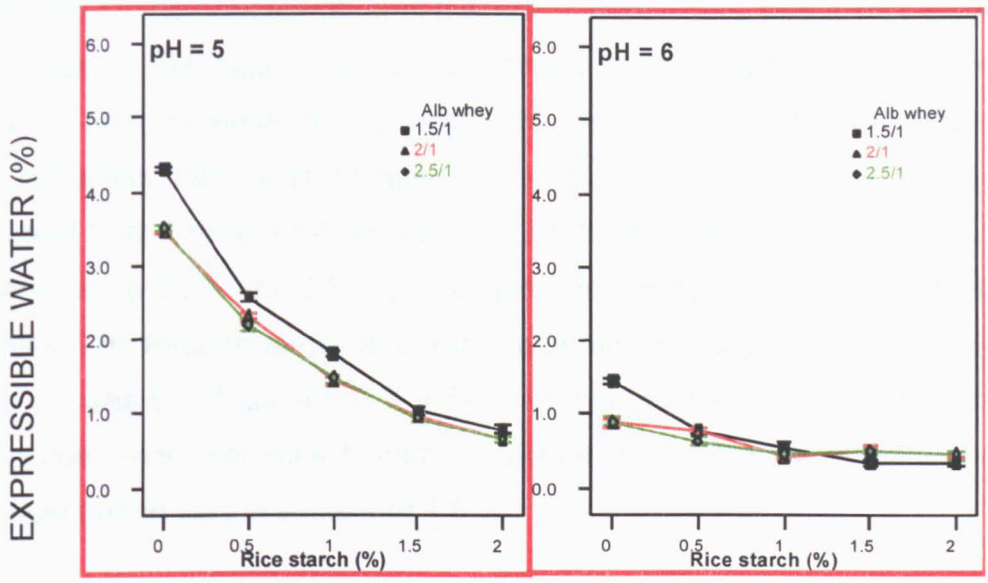


Figure 4.6-D

The Effect of Rice Starch on Expressible Water



The observation in Figs. 4.6-C and 4.6-D suggests that the pores in the gels of lower concentration of rice starch are constructed in such a way that more water is admitted into the interstices. As the concentration is increased, there is tendency towards a tighter, more cohesive structure allowing for less water ingress and less water exudation when the gel network is disrupted with the applied pressure. **Do the rice starch molecules modify the gel in some way as to create smaller pores or do they act by a different mechanism perhaps as an active filler and bind more of the available water themselves?** The experiment by Aguilera & Baffico (1997) using rice starch provides some insight. They proved that in heat-induced gel of whey protein and cassava starch, the starch granules swelled rapidly and remained distinctive as swollen granules in the mixed matrix. The conclusion was that the starch granules actively participate in the gel structure by removing water. They further proposed a new term of 'active phase-separated gel' to distinguish this phenomenon from the nearly phase separated gels that are formed due to thermodynamic inactivity.

Fracture Properties

The fracture properties of the gel did not appear to be tremendously affected by the concentration of the rice starch. There was a very small increase in the magnitude of the fracture stress, confirming that rice starch can increase the strength of the gel, at both pH levels. Nonetheless, there was a bigger influence from the binder type as was evident from Figure 4.6-F with binder 2.5/1. At pH 5.0 and with the rice starch up to 0.5%, the 2.5/1 gel sample had a reduced value of fracture stress. The fracture stress and fracture strain were slightly higher when the pH was 6.0 (Figs. 4.6-E & 4.6-F). At this pH, the fracture stress for all three binder samples became resistant to the rice starch once the concentration was in excess of 1.0%.

Figure 4.6-E

The Effect of Rice starch on Fracture Strain

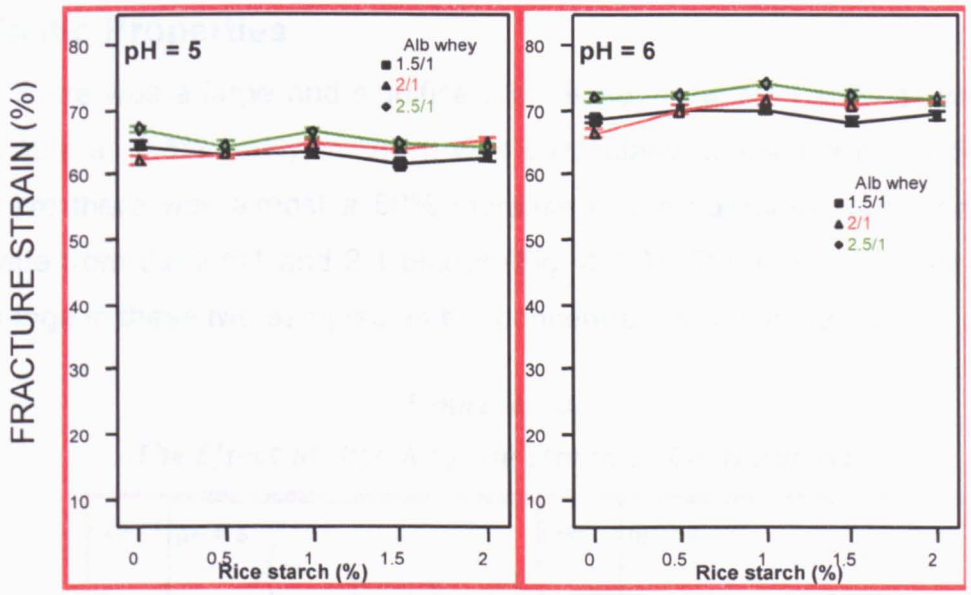
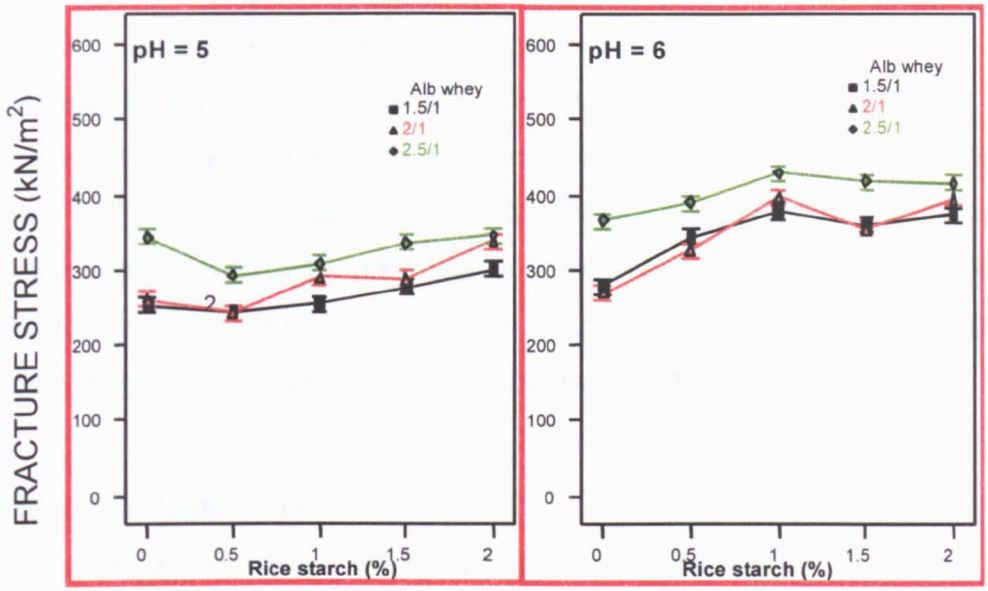


Figure 4.6-F

The Effect of Rice Starch on Fracture Stress



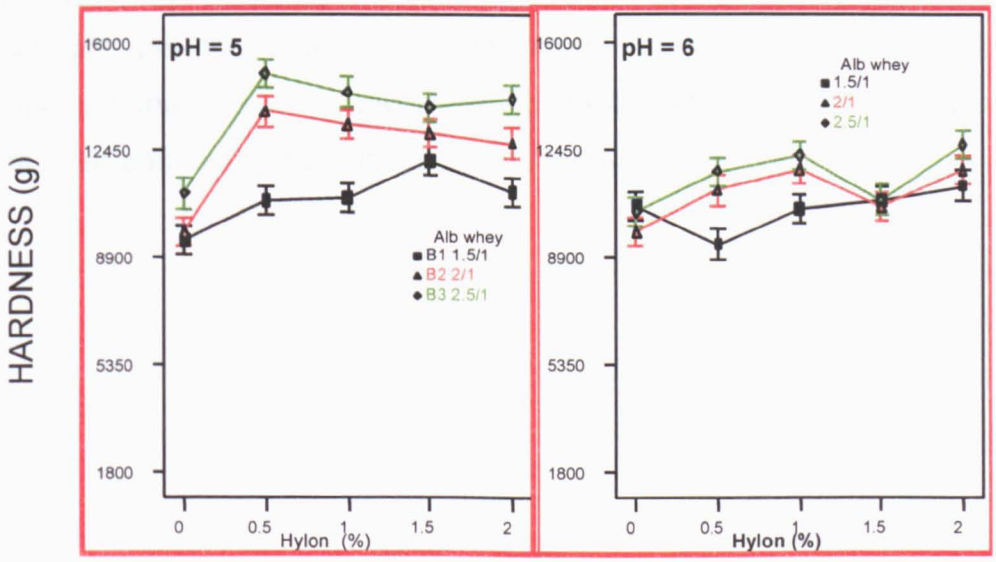
4.7 The Effect Of High Amylose Starch (Hylon VII)

Elastic Properties

There was a large and significant increase in the gel hardness with as little as 0.5% of Hylon VII. It was particularly noticeable at pH 5.0 where there was almost a 50% increase in the hardness of the gels made from the 2.5/1 and 2/1 binder (Fig. 4.7-A). There was no further change in these two samples as the concentration was increased.

Figure 4.7-A

The Effect of High Amylose Starch on Gel Hardness



At pH 6.0, the change in gel hardness for all gel samples was rather more sedate but was still significantly higher for the 2.5/1 and 2/1 binder at low concentrations of the high amylose starch (0.5-1.0%). With the exception of the 1.5/1 binder, the amylose starch conferred increased hardness to the gel samples at pH 5.0 than at pH 6.0.

The observation from the present study is in agreement with the work of Shao *et al.* (2006) and Sandhu & Singh (2007) who concluded that starches with high amylose content tend to spawn harder gels especially when they have a high degree of polymerisation, longer chain length and smaller number of chains per molecule.

Plastic Properties

The high amylose starch hardly had any effect on the gel springiness. However when gel samples made at pH 6.0 were compared to those made at pH 5.0, the former showed higher springiness values (Fig. 4.7-B). At pH 5.0, the gel springiness varied directly with the amount of albumen in the binder because the springiness of the gel rose as the proportion of albumen was increased. The effect of added albumen in gel springiness was not so obvious at pH 6.0 as it was at pH 5.0.

Like rice starch, the high amylose starch reduced the viscous properties of the gel and was evident by the reduction in the *a*-value of the gel as shown in Fig. 4.7-G. It tallied with the increase in gel hardness. At pH 5.0, there was a separation between the plot for the 1.5/1 gel and the other two. The higher *a*-value for this sample, suggested that the whey protein concentrate did not engender inter-molecular connections as effectively as the albumen.

Figure 4.7-B

The Effect of High Amylose Starch on Gel Springiness

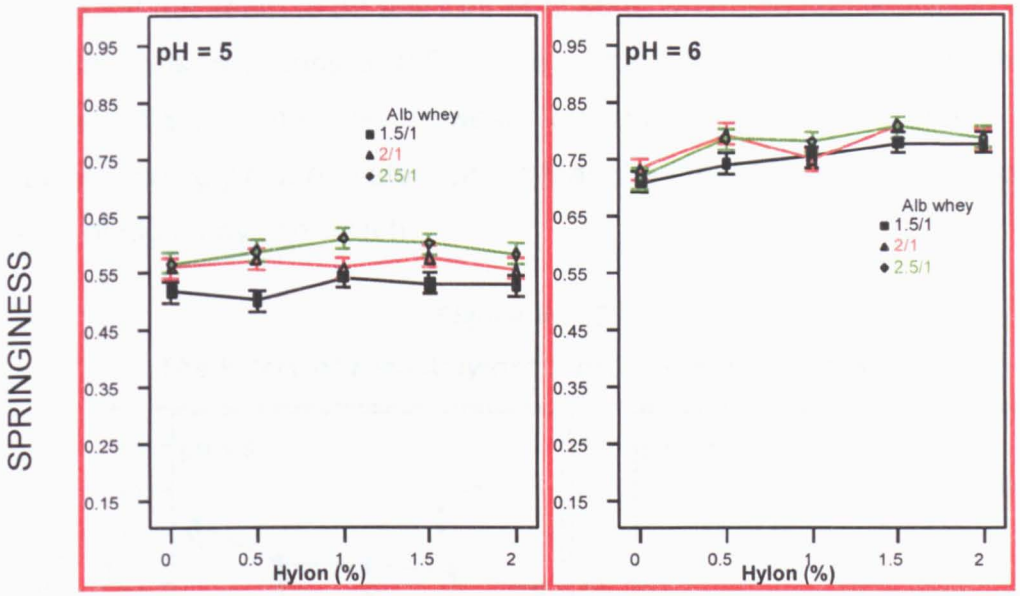
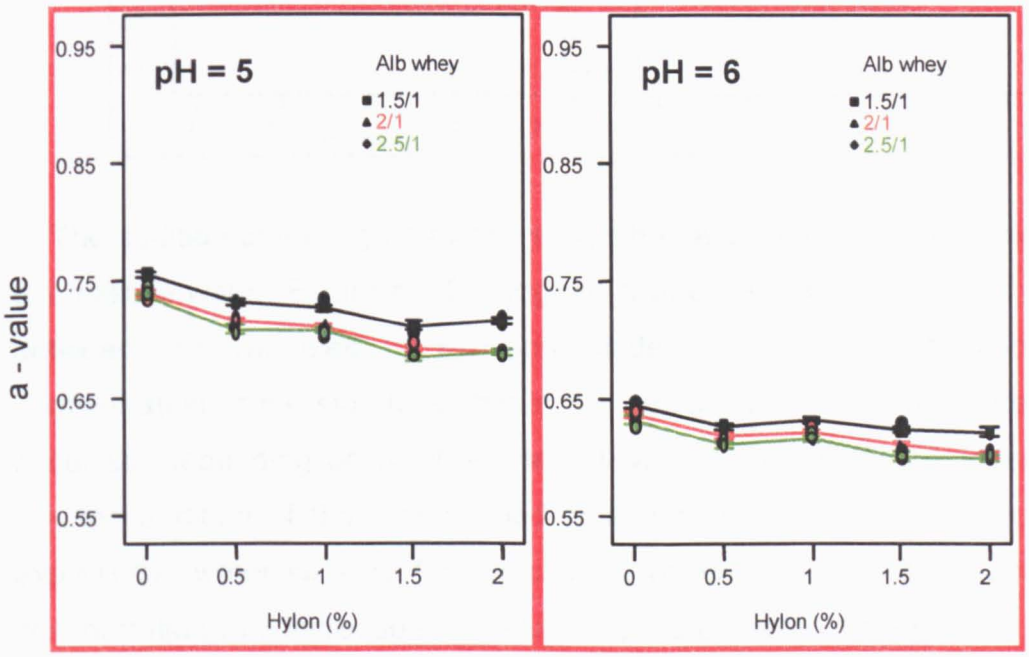


Figure 4.7-G

The Effect High Amylose Starch on a - Value

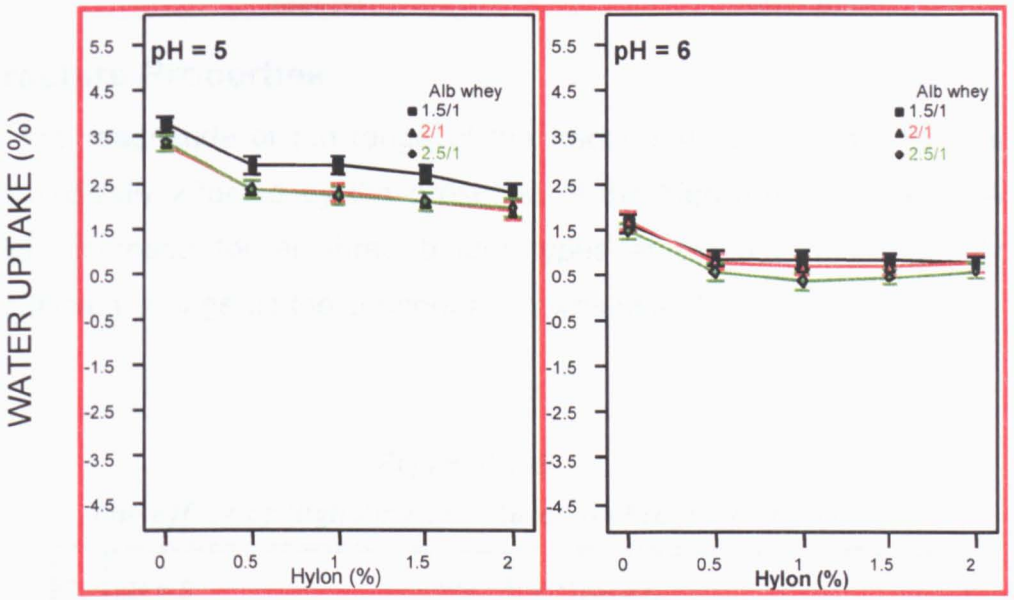


Microstructure

Water uptake was only slightly affected by the addition of the high amylose starch (Fig. 4.7-C). The effect was evinced by a slight drop in the water uptake with a 0.5% introduction of the starch at either pH. There did not seem to be any further change when the concentration of the starch is in excess of 0.5%. It is worth noting that at pH 5.0, the binder 1.5/1, with the lowest amount of albumen, had a correspondingly higher water uptake than the other two binders at each level of high amylose starch.

Figure 4.7-C

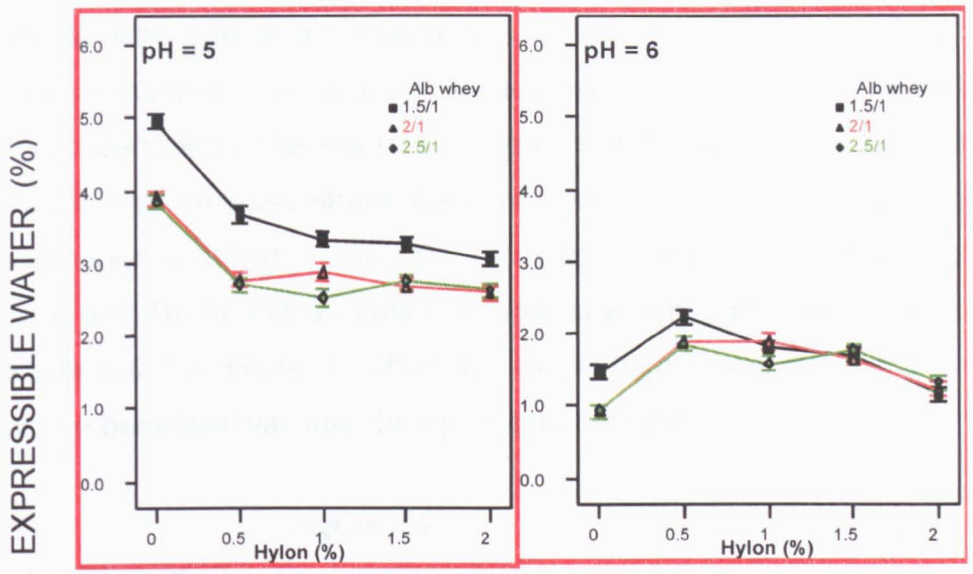
The Effect of High Amylose Starch on Water Uptake



The addition of the high amylose starch had a profound effect on the expressible water. Figure 4.7-D suggests that there was an interaction between the variables of pH and binder type. At up to 0.5% concentration of the starch, a shift in the amount of expressible water occurred, depending on whether the pH was 5.0 or 6.0. At the lower pH, the addition of 0.5% starch led to a reduction in the amount of expressible water with no further change observed when the starch concentration was increased. However at pH 6.0, there was a small but significant increase in the expressible water followed by a gentle decline, as the concentration was increased further.

Figure 4.7-D

The Effect of High Amylose Starch on Expressible Water

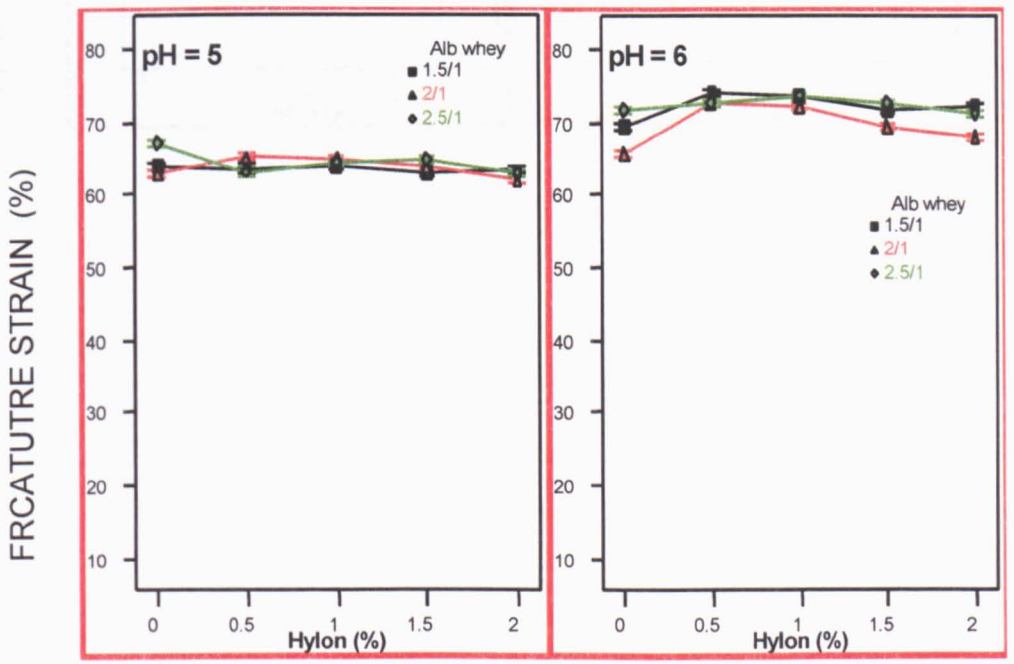


Fracture Properties

The magnitude of the range of the fracture strain (Fig. 4.7-E) was not radically affected by the presence of the high amylose starch. A slight increase for all three binder types at pH 6.0 was the only significant change as the concentration was raised to 0.5%.

Figure 4.7-E

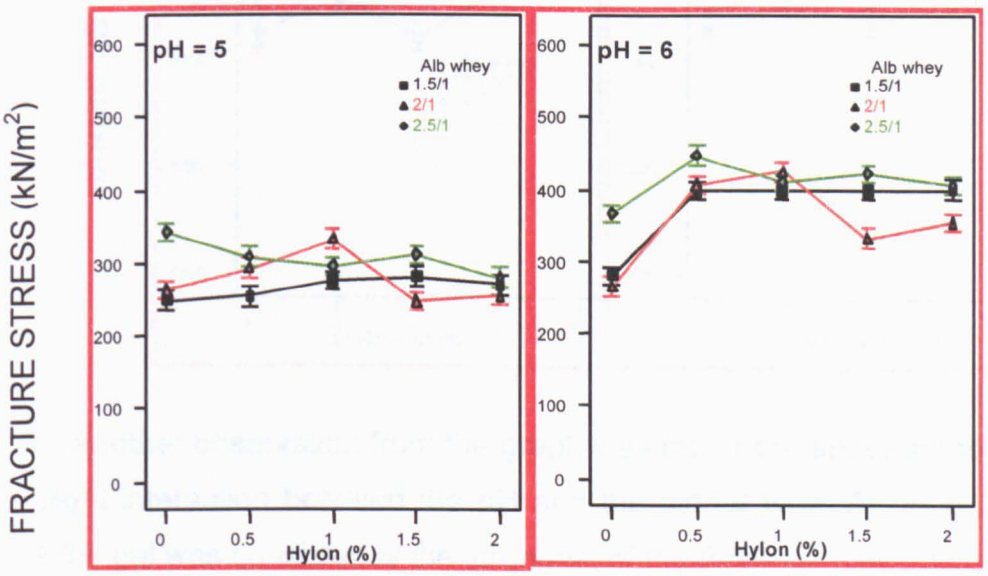
The Effect of High Amylose Starch on Fracture Strain



There was a slight increase in the fracture stress with the addition of 0.5% of the starch at pH 6.0 (Fig. 4.7-F). No further change was observed with increased concentration. The 2/1 gel sample behaved in a peculiar manner. After a small, steady rise in fracture stress at up to 1.0% concentration, there was a sharp drop in fracture stress between 1.0-1.5% high amylose starch concentration. It is noteworthy that this phenomenon occurred in almost exactly the same manner at both pH 5.0 and 6.0. There was an optimum level at which high amylose starch interacts with the binder to affect the gel strength most beneficially. It would be beneficial validate the optimum at pH 6.0.

Figure 4.7-F

The Effect of High Amylose Starch on Fracture Stress



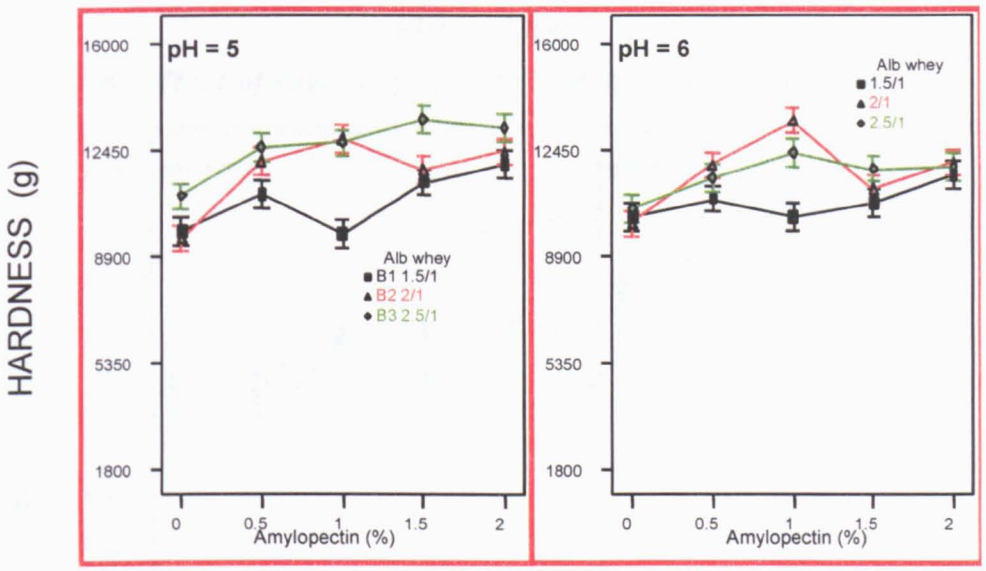
4.8 The Effect Of High Amylopectin Starch (Novation™ 2700)

Elastic Properties

The addition of high amylopectin starch to the gel system gently increased the hardness of all the gel samples (Fig. 4.8-A). The change in gel strength was particularly evident at pH 5.0.

Figure 4.8-A

The Effect of High Amylopectin Starch on Gel Hardness



Another observation from the graph was that there appeared to be a slight interaction between the pH and the binder type. At pH 5.0, the 2.5/1 gel was consistently the strongest of the three gel types across all concentration values of the high amylopectin starch. At pH 6.0, there was an inversion of the linear plots with the 2/1 gel sample, in the main, becoming the stronger gel. The interaction appeared to be strongest at a concentration of 1.0 – 1.5% amylopectin.

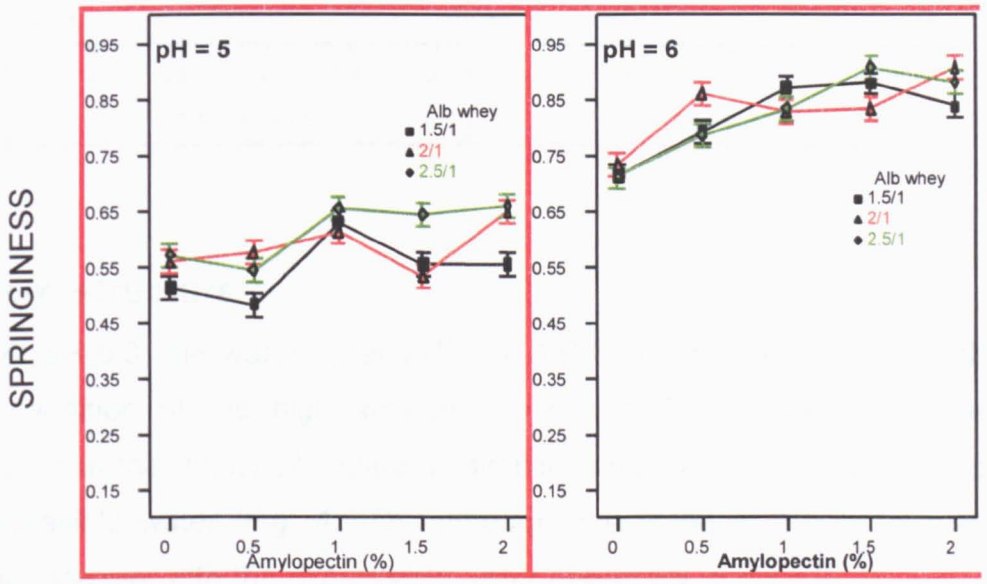
Interestingly at a concentration of 1.5% of the high amylopectin starch, the hardness of the 2/1 binder sample dropped at both pH 5.0 and 6.0.

Plastic Properties

The springiness of the gel with added high amylopectin starch increased steadily with increasing concentration of the starch at pH 6.0 but binder type seemed to have little effect (Fig. 4.8-B). At pH 5.0, there was an initial lag in reaction at up to 0.5% concentration. This was followed by a step change between 0.5 and 1.0%. Above 1.5% there was either no further change or a slight drop. The magnitude of the change was more pronounced at pH 6.0 but there were no major differences in the binder type.

Figure 4.8-B

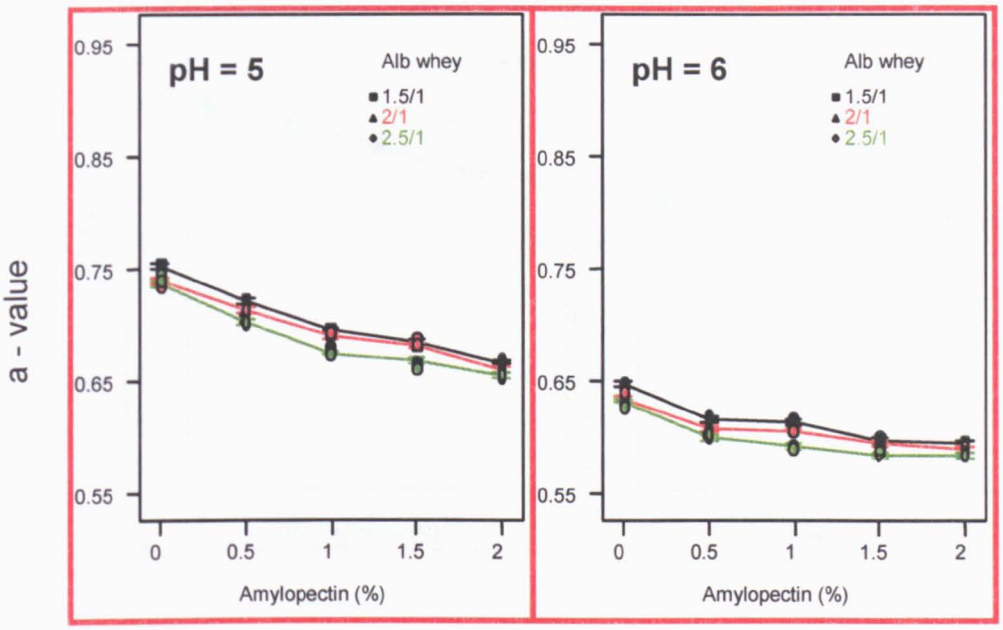
The Effect of High Amylopectin Starch on Gel Springiness



The *a*-value of the gels was reduced as the high amylopectin starch content was raised (Fig. 4.8-G). The implication of a reduction in *a*-value is the improved inter-molecular connections manifested in increased elastic properties and reduced viscous properties as shown by the gel hardness.

Figure 4.8-G

The Effect High Amylopectin Starch on a- Value



Micro-structure

At pH 5.0, the water uptake (Fig. 4.8-C) was steadily reduced with the addition of the high amylopectin starch. There was very little change at the higher pH value. A similar trend was observed with the expressible water (Fig. 4.8-D), although, the slope of the curve was much steeper with the expressible water especially at pH 5.0.

Figure 4.8-C

The Effect of High Amylopectin Starch on Water Uptake

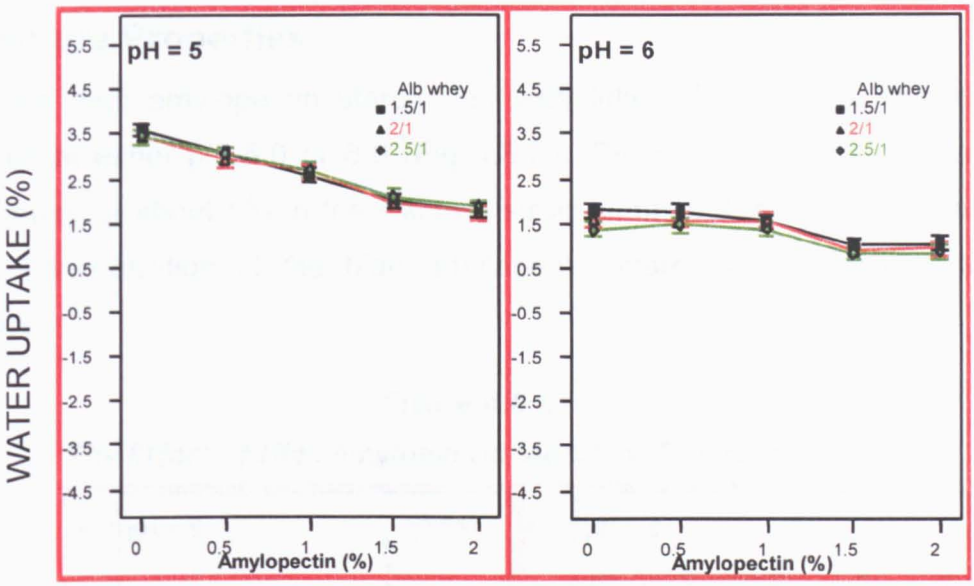
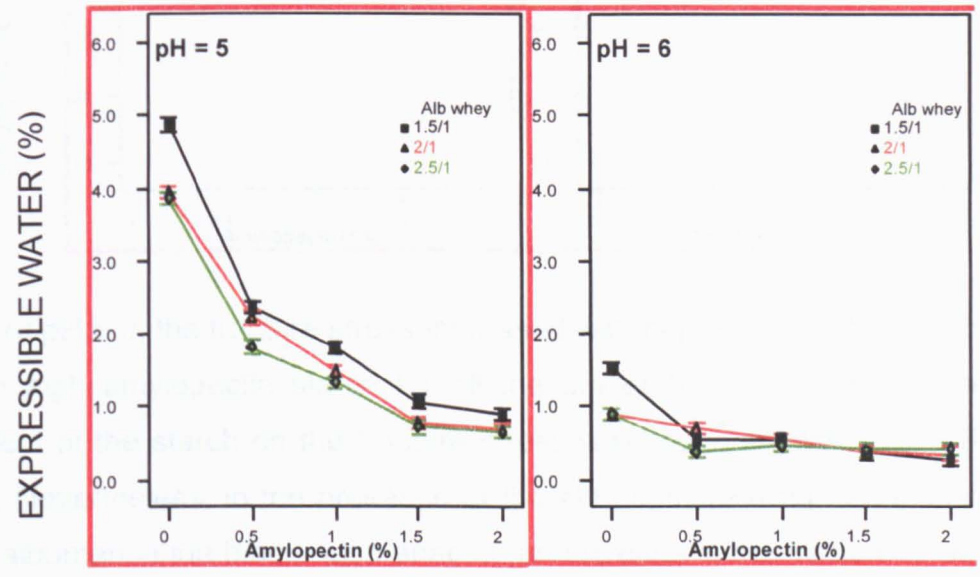


Figure 4.8-D

The Effect of High Amylopectin on Expressible Moisture



As there were only small differences in the magnitude of the water uptake and the expressible water with changes in the binder type at each level of the high amylopectin starch, it can be concluded that the binder type, in the presence of high amylopectin starch and within the

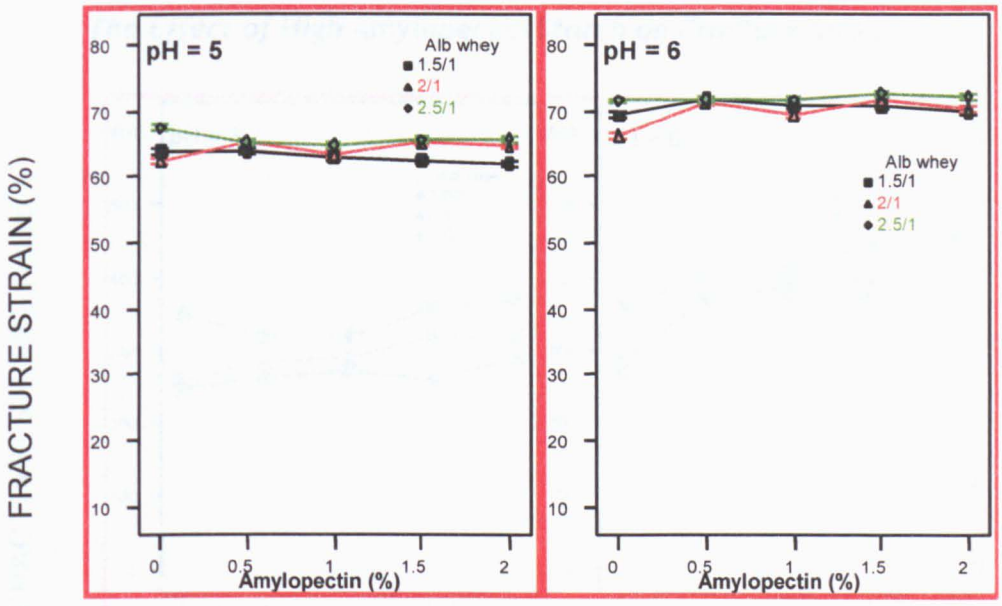
range of the experimental data, did not significantly affect the microstructure.

Fracture Properties

The high amylopectin starch had very little effect on the fracture strain at either pH 5.0 or 6.0 (Fig 4.8-E). The exception was a small increase of about 5% in the fracture strain at pH 6.0 in the 2/1 gel as the concentration of the high amylopectin starch was increased to 0.5%.

Figure 4.8-E

The Effect of High Amylopectin Starch on Fracture Strain



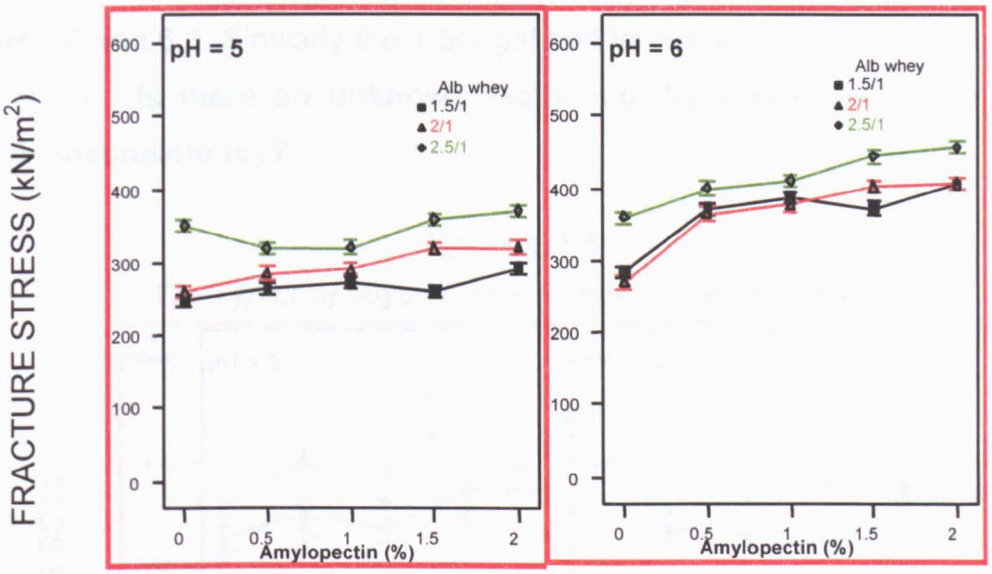
At pH 6.0, the fracture stress increased with higher concentrations of the high amylopectin starch for all the binder types. At pH 5.0, the effect of the starch on the fracture stress was much smaller (Fig. 4.8-F). Nevertheless, in the presence of the starch, increasing the amount of albumen in the binder also enabled an increase in the fracture stress especially with binder 2.5/1 which had the highest amount of albumen.

Unlike the high amylose starch in which a peak in fracture stress occurred at about 0.43 Nmm^{-2}, high amylopectin starch concentrations varied directly with the gel strength, as shown by the constantly increasing fracture stress.

Olsson *et al.* (2000) performed a series of experiments with β-lactoglobulin and they incorporated potato amylopectin at various levels. They found out that at lower concentration (<0.5%) the fracture stress was constant. At concentrations in excess of 0.5%, the stress fracture decreased drastically for high viscosity amylopectin but increased with low viscosity amylopectin. Olsson *et al.* hypothesized that this change was either due to a change in the aggregation mechanism or phase separation. Light microscopy confirmed that amylopectin caused the protein aggregates to become larger and thicker and that the resulting gel had larger pores.

Figure 4.8-F

The Effect of High Amylopectin Starch on Fracture Stress



In another study, Olson *et al.* (2003) carried out a test of β-lactoglobulin gel (6%) with varying concentrations of amylopectin with molecular weight ranging from 24000 – 44000 kDA. The conclusion was that the differing molecular weights of the amylopectin altered the mobility of the β-lactoglobulin clusters leading to a variation in pore size and the resultant molecular structure of the gel. The gel containing 0.75% of 24000 kDA amylopectin had an inhomogeneous microstructure with a mixture of both small and large pores whilst the gel containing 2% of the 44000 kDA amylopectin consisted of mostly

larger pores. When a fracture test was carried out as part of the experimentation, the gel containing 2.0% of 24000 kDA amylopectin had double the stress at fracture as compared to the gel containing 0.75% 44000 kDA amylopectin.

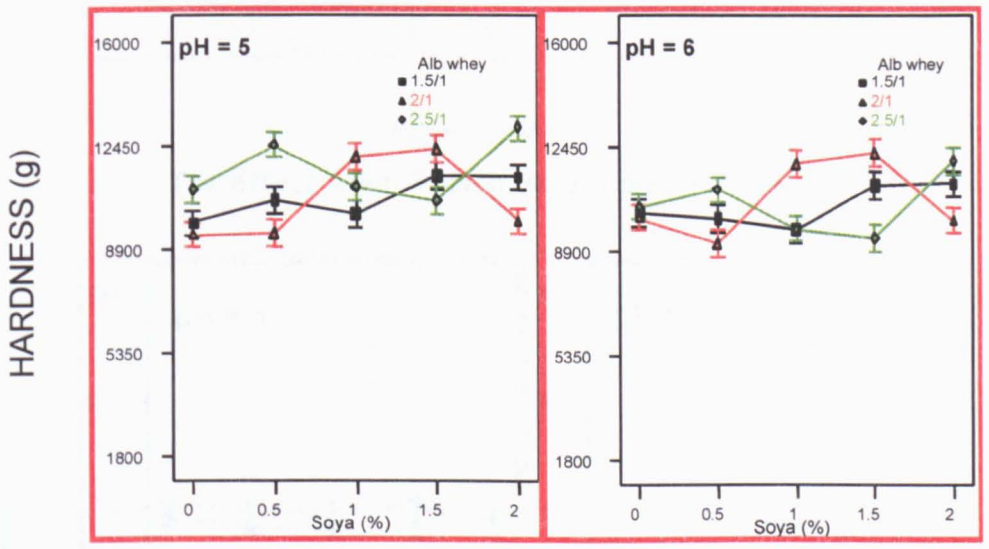
4.9 The Effect Of Soya Isolate

Elastic Properties

The effect of soya isolate on the gel hardness (Fig. 4.9-A) was at best mixed. The pattern was consistent at the different pH values but not with the different binders. The 2/1 binder showed maximum hardness at a concentration of 1.0–1.5% for both pH values. On either side of this maximum, there was a drop in gel hardness. On the other hand, the 2.5/1 gel exhibited minimum hardness at 1.0–1.5% at both pH 5.0 and 6.0. Similarly the 1.5/1 gel had its maximum gel strength at 1.5–2%. **Is there an unknown factor e.g. hydration that caused this inconsistency?**

Figure 4.9-A

The Effect of Soya Protein Isolate on Gel Hardness



Plastic Properties

Like gel hardness, the changes in springiness were small but seemingly inconsistent (Fig. 4.9-B). The change in the springiness was more dependent on the pH than the addition of the soya isolate or the binder composition, although at pH 5.0, the 2.5/1 binder gel was the springiest. The springiness peaked at 1.0–1.5% soya isolate concentration.

Figure 4.9-B

The Effect of Soya Protein Isolate on Gel Springiness

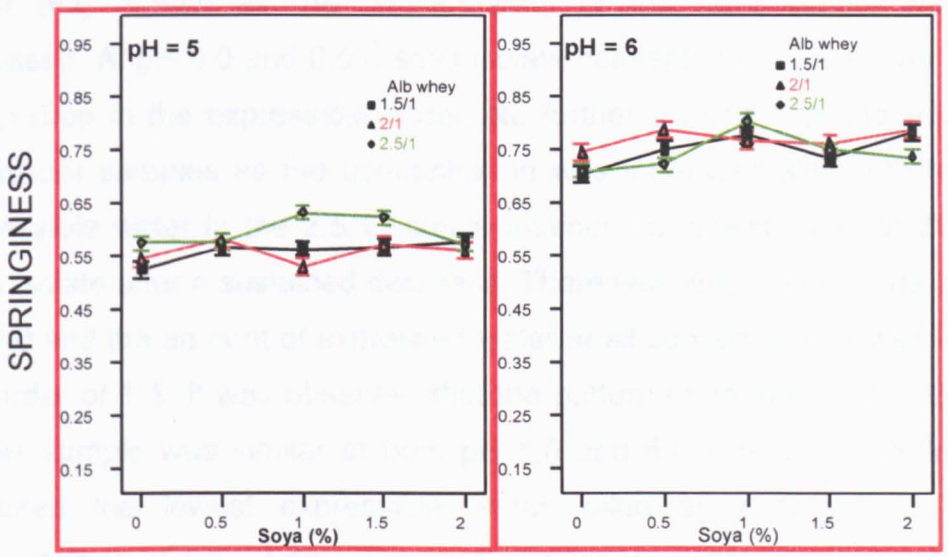
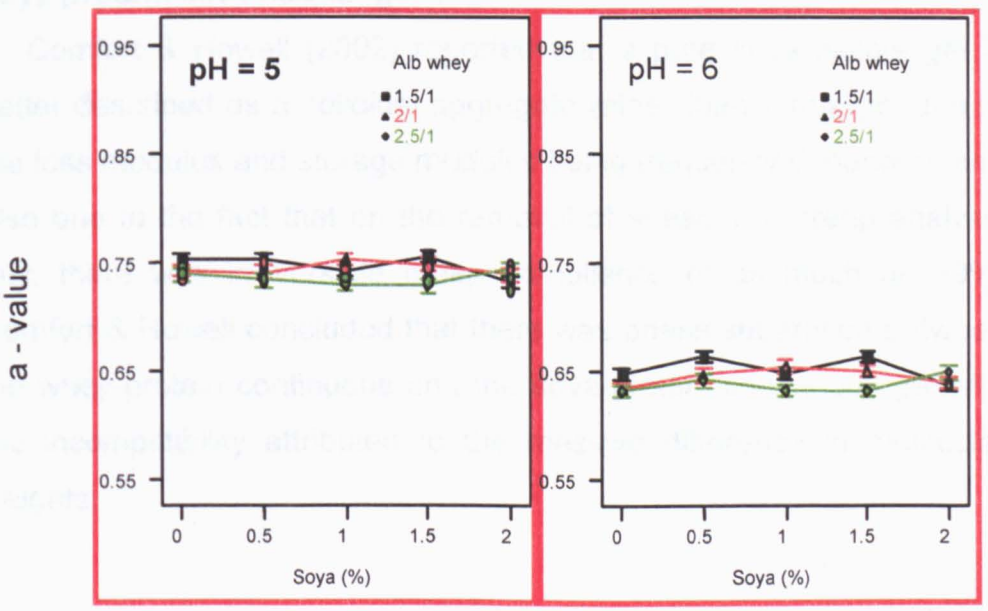


Figure 4.9-G

The Effect Soya Protein Isolate on a - Value



There is hardly any change in the *a*-value in the presence of soya isolate. There was a bigger effect of the pH on the *a*-value over an above the effect of the concentration of the soya protein isolate.

Microstructure

Just like some of the other attributes, water uptake (Fig. 4.9-C) was not affected by the amount of soya isolate in the gel. The pH had a bigger effect.

The biggest observable change was in the reduction of expressible water (Fig. 4.9-D) as the concentration of the soya isolate was increased. At pH 5.0 and 0.5% soya isolate concentration, there was a sharp drop in the expressible water. No further change took place in the binder samples as the concentration was increased although the expressible water in the 2.5/1 sample attained its lowest value at 2% soya isolate after a sustained decrease. There was very little change at pH 6.0 and the amount of expressed water at all concentrations was of the order of 1%. It was observed that the pattern of change in the 2/1 binder sample was similar at both pH 5.0 and 6.0 where this binder exhibited the lowest expressible water value at a soya isolate concentration of 1.0 – 1.5%. Increasing the isolate to 2% then led to an increase in the expressible water, again at both pH values. **Is this evidence of a change from a whey/albumen continuous gel to a soya protein continuous 'gel'?**

Comfort & Howell (2002) reported that a pure soya isolate gel is better described as a colloidal aggregate rather than a true gel due to the loss modulus and storage modulus being frequency dependent and also due to the fact that on the removal of stress in a creep analysis test, there was irreversible creep compliance of as much as 89%. Comfort & Howell concluded that there was phase separation between the whey-protein continuous and the soya-protein continuous gel with the incompatibility attributed to the massive difference in molecular weights.

Figure 4.9-C

The Effect of Soya Protein Isolate on Water Uptake

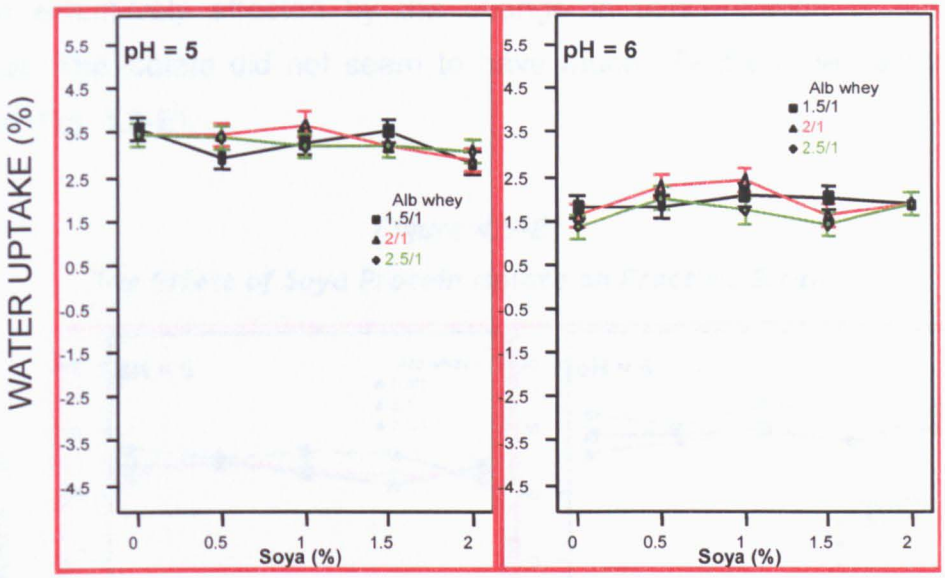
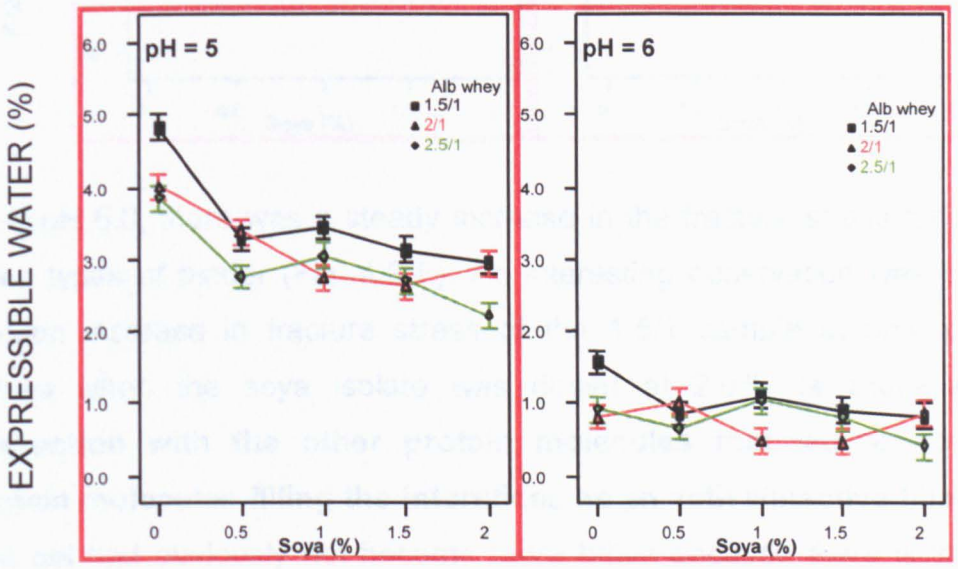


Figure 4.9-D

The Effect of Soya Protein Isolate on Expressible Water

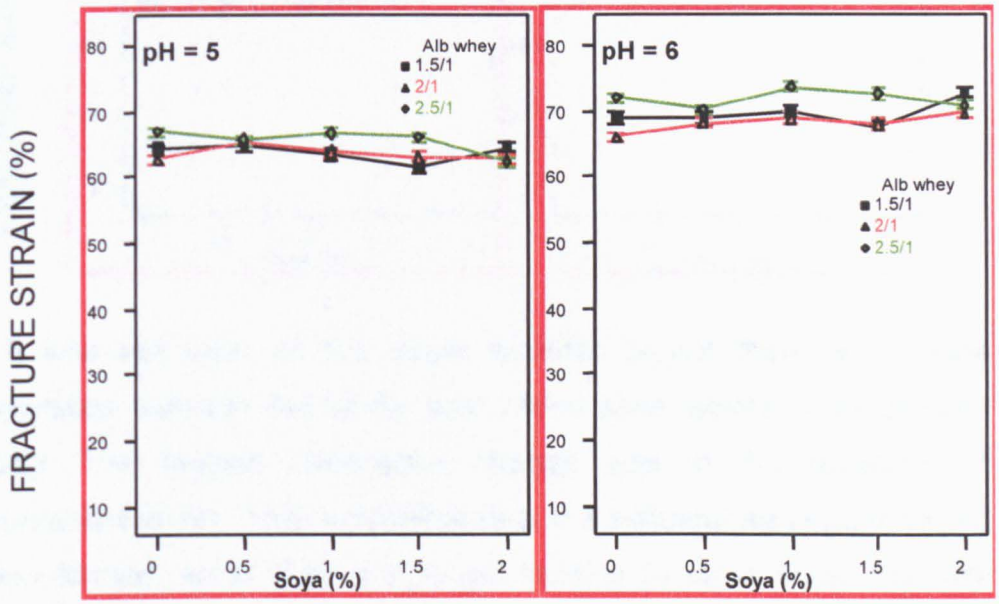


Fracture Properties

Of the two fracture properties measured, the fracture stress was more extensively affected by the change in concentration of soya isolate. The isolate did not seem to have much effect on the fracture strain (Fig. 4.9-E).

Figure 4.9-E

The Effect of Soya Protein Isolate on Fracture Strain

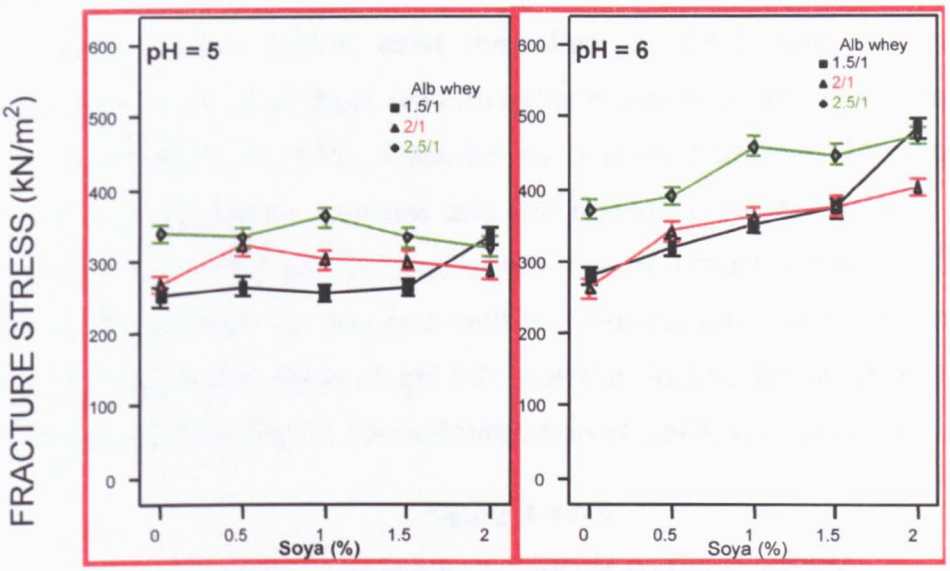


At pH 6.0, there was a steady increase in the fracture stress for all three types of binder (Fig. 4.9-F). An interesting observation was the sudden increase in fracture stress of the 1.5/1 sample at both pH values when the soya isolate was dosed at 2.0%. **Is there an interaction with the other protein molecules that led to soya protein molecules filling the interstices as an active/inactive filler?** The gel had obviously not become more brittle because there is little effect on the fracture strain.

The fracture stress was not affected much by the soya isolate at pH 5.0. However all three binders had the same nominal fracture stress at 2.0% concentration of the soya isolate.

Figure 4.9-F

The Effect of Soya Protein Isolate on Fracture Stress



It was not clear at this stage whether or not there was phase separation between the binder and added soya isolate in the present study. The biggest observable change was in the reduction of expressible water. This is possibly due to a colloidal aggregate having been formed rather than a true gel leading to large pores and low interstitial capillary pressure (Comfort & Howell, 2002). It may also be the reason for the increase in fracture stress with increasing soya isolate concentration. Increasing fracture stress is generally indicative of a gel that does not fracture easily and may be due to the fact that the aggregates or network are more flexible and can move easily in the viscous phase (Olsson *et al.*, 2000) as opposed to the elastic phase.

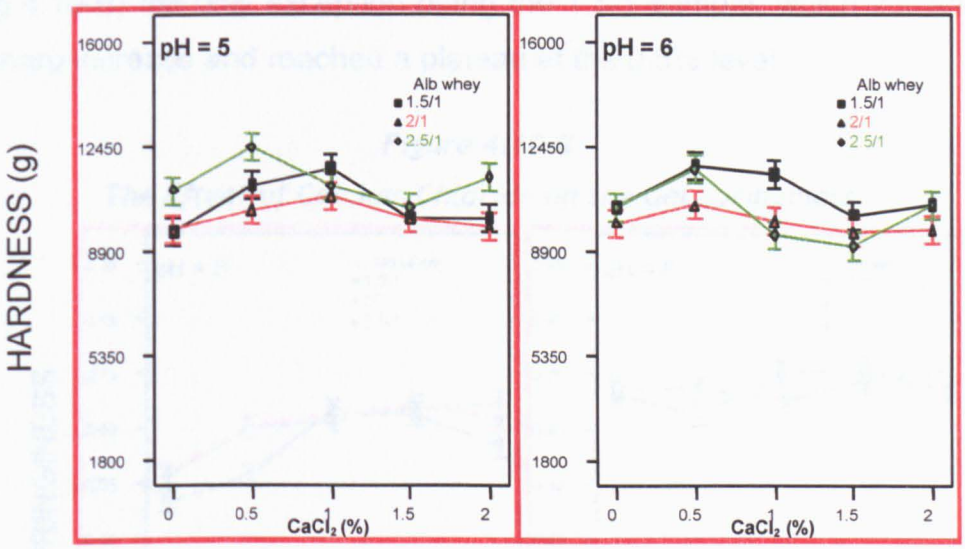
4.10 The Effect Of Calcium Chloride ($CaCl_2$)

Elastic Properties

Figure 4.10-A shows what the effect of $CaCl_2$ was on the gel hardness. At pH 5.0, there was an initial increase in gel hardness up to a concentration of 0.5%, followed by a steady decline. The point at which the alteration occurred and the decline commenced was 1.0% $CaCl_2$ for the 1.5/1 and 2/1 binder but it started much earlier (0.5%) for the 2.5/1 sample, *i.e.* the one with the highest amount of albumen. A similar effect was seen at pH 6.0, but the decline for all three binder samples started after 0.5% concentration of $CaCl_2$ was achieved.

Figure 4.10-A

The Effect of Calcium Chloride on Gel Hardness



There is considerable confusion with regards to the role of calcium and its effect in a whey protein gel. Lupano *et al.* (1992) showed in a study with whey protein isolate that at low levels (<0.02M) calcium enhances protein-protein interactions and contributes positively to the gel network but that when the calcium is above this level, it causes excessive protein aggregation with detrimental effects on gel hardness. However, Beaulieu *et al.* (2001) reported that whey protein gels made with 0.01-0.02M calcium have particulate microstructures and low water holding capacity. Hongsprabhas *et al.* (1999) suggested that in

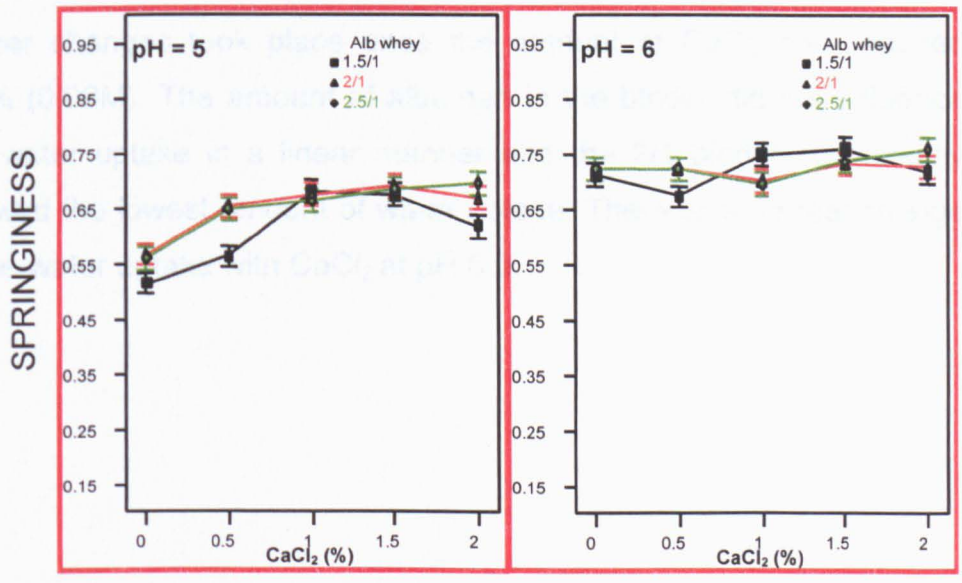
calcium-induced whey protein gelation, 0.03 – 0.04M $CaCl_2$ was the turning point at which the gel structure changed. Below this concentration, the charge screening mechanism dominated but above the 0.04M, the cross-linking mechanism gained ascendancy. The mechanism described by Hongsprabhas *et al.* (1999) may well be partly or totally responsible for the dip observed in gel hardness in the present study at 0.5 – 1.0% $CaCl_2$ concentration.

Plastic Properties

There was a small increase in the gel springiness at low levels of $CaCl_2$ observed at pH 5.0 but the effect appears to have been completed by the time the concentration reaches 1.0% (0.09M). At pH 6.0, there was no major change with increasing concentration of $CaCl_2$ (Fig.4.10-B) the only exception being the 1.5/1 sample, which showed a sharp increase and reached a plateau at the 0.5% level.

Figure 4.10-B

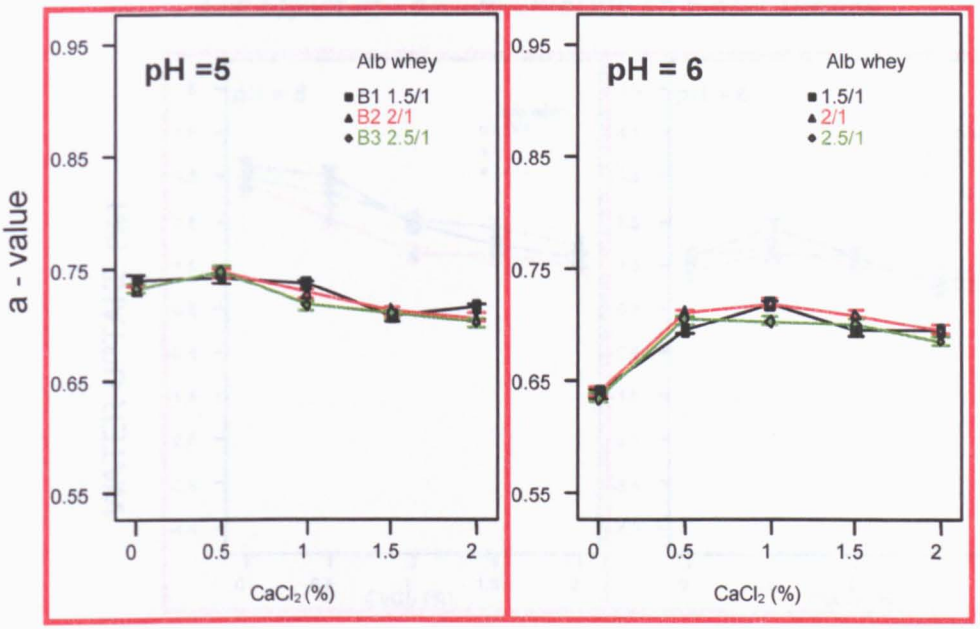
The Effect of Calcium Chloride on the Gel Springiness



The sharp increase in *a*-value at pH 6.0 with the introduction of 0.5% $CaCl_2$ (Fig. 4.10-G), is symptomatic of an increase in the viscous properties of the gel. Beyond this point, there was no further increase and the *a*-value stayed constant. At pH 5.0, the opposite takes place and added $CaCl_2$ reduced the viscous properties of the gel.

Figure 4.10-G

The Effect of Calcium Chloride on a - Value

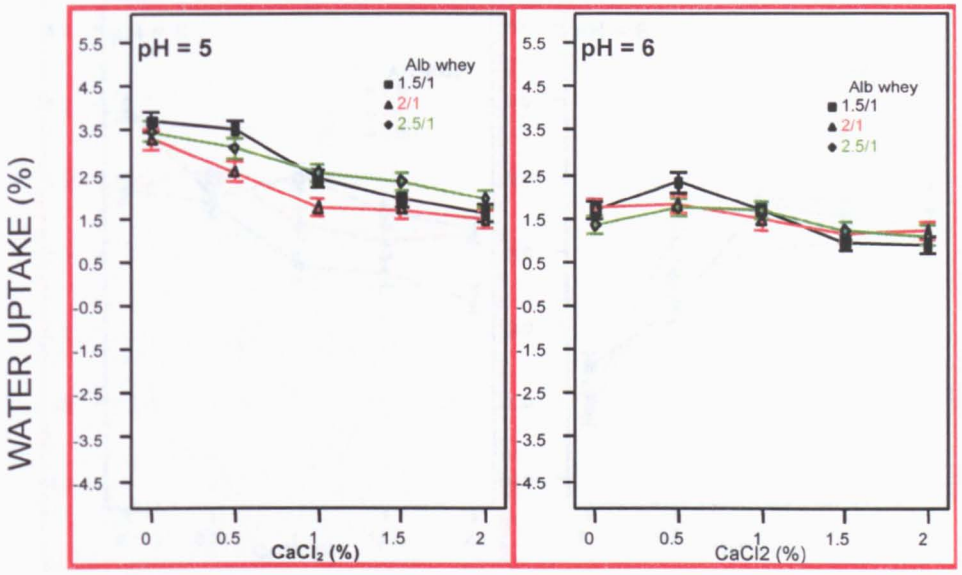


Micro-structure

Figure 4.10-C shows that the presence of $CaCl_2$ led to a steady reduction in the water uptake in all three binder samples at pH 5.0. No further changes took place once the amount of $CaCl_2$ had reached 1.0% (0.09M). The amount of albumen in the binder did not influence the water uptake in a linear manner and the 2/1 binder consistently showed the lowest amount of water uptake. There was no real change in the water uptake with $CaCl_2$ at pH 6.0.

Figure 4.10-C

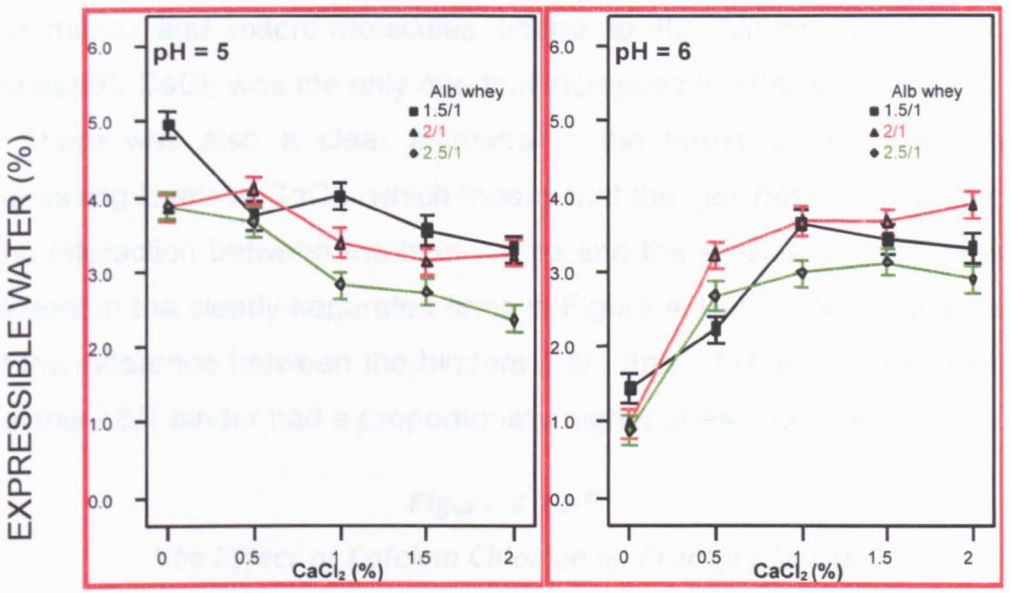
The Effect of Calcium Chloride on Water Uptake



Unlike the water uptake, there was considerable change to the expressible water when $CaCl_2$ was introduced to the gel system. Evidence of interaction exists between pH and the binder type as evinced by the crossover of the lines in the Figure. 4.10-D. Expressible water was highest at pH 5.0 with no added $CaCl_2$ and lowest at pH 6.0, again with no added $CaCl_2$. When the concentration of the $CaCl_2$ was increased at pH 5.0, the effect was to gradually reduce the amount of expressible water with a trough occurring at about 1.0–1.5% concentration of $CaCl_2$. On the other hand when the amount of $CaCl_2$ was increased at pH 6.0, there was a dramatic increase in the expressible water at 0.5%, with additional $CaCl_2$ not bringing about any further increase. Unlike the situation at the lower pH value, the 2/1 gel had the higher amount of expressible water at virtually all the levels of $CaCl_2$ tested at pH 6.0.

Figure 4.10-D

The Effect of Calcium Chloride on Expressible Water

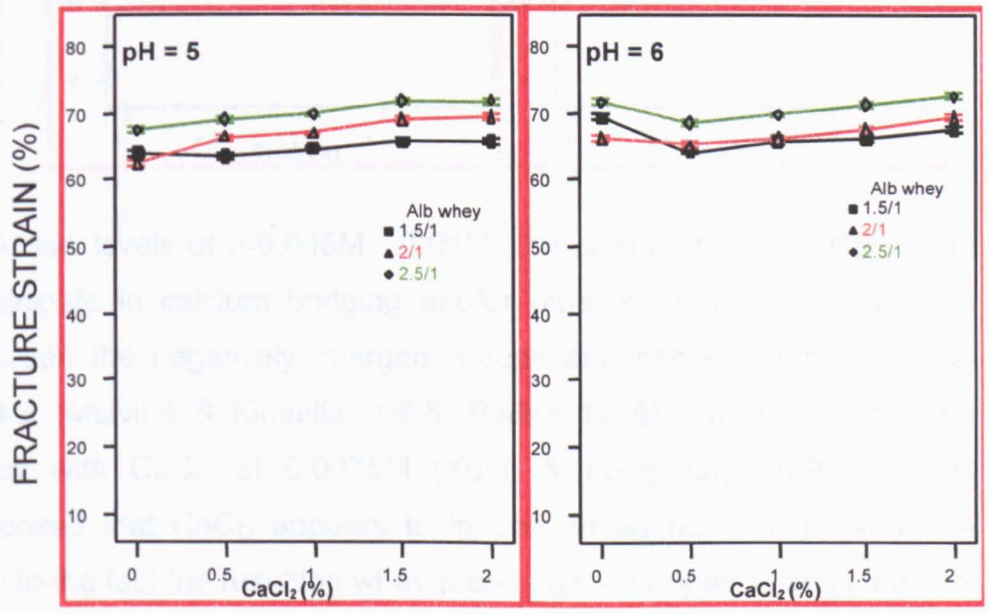


Fracture Properties

Although the increase in the fracture strain with added $CaCl_2$ was very small, there was a significant difference in fracture strain with the various binders observable at both pH values (Fig. 4.10-E).

Figure 4.10-E

The Effect of Calcium Chloride on Fracture Strain

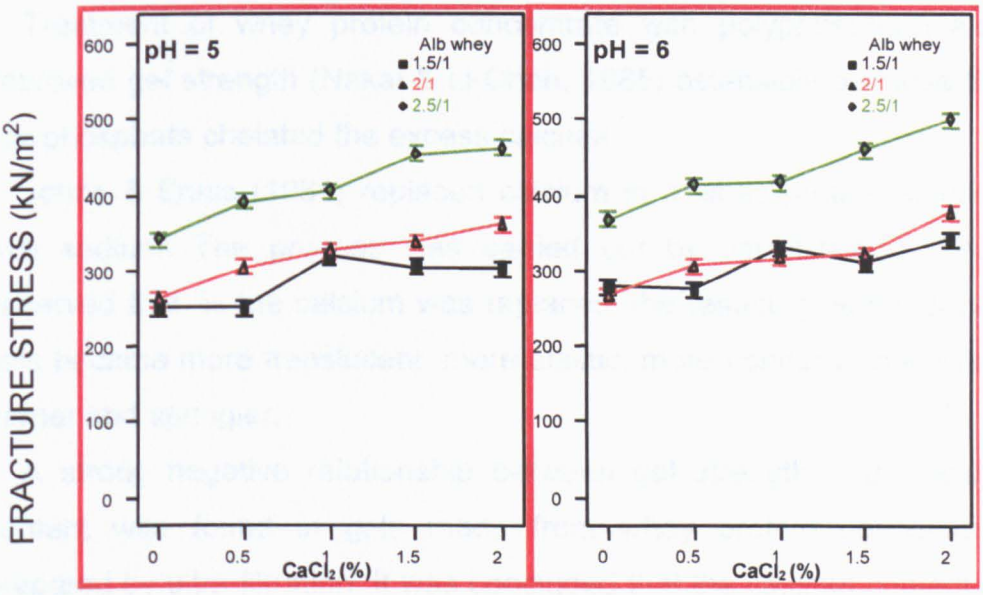


As the amount of albumen in the binder was increased, so did the fracture strain, implying that albumen in conjunction with the $CaCl_2$ reduces the brittleness (or increases the toughness) of the gel. Of all the micro- and macro-molecules added to the binder system and assessed, $CaCl_2$ was the only one that increased the fracture strain.

There was also a clear increase in the stress at fracture with increasing levels of $CaCl_2$, which means that the gels became stronger. The interaction between the binder type and the amount of $CaCl_2$ was evident in the clearly separated lines in Figure 4.10-F. The magnitude of the difference between the binders 1.5/1 and 2.0/1 was quite small, but the 2.5/1 binder had a proportionally higher stress fracture.

Figure 4.10-F

The Effect of Calcium Chloride on Fracture Stress



At low levels of (<0.005M - 0.01M) the calcium from the $CaCl_2$ can participate in calcium bridging and/or other electrostatic interactions between the negatively charged groups and can strengthen the gel matrix (Mulvihill & Kinsella, 1988; Barbut 1995). However in another study with $CaCl_2$ at 0.0075M (Kuhn & Foegeding, 1991), it was theorized that $CaCl_2$ appears to induce an aggregated gel structure due to the fact the resulting whey protein gel was opaque and curd-like. It was also reported in the same study that increasing the amount of

$CaCl_2$ up to 0.2-1.0M then caused a sharp increase in the shear stress (Kuhn & Foegeding, 1991).

In the experiment by Barbut (1995) with calcium at 0.025 M, the water holding capacity of the resultant whey gel was poor.

Kuhn & Foegeding (1991) were able to show that $CaCl_2$ effected a rapid increase in both stress and strain at failure up to about 0.0075M after which there was no further change in the stress or strain.

Schmidt *et al.* (1979) reported that in a 10% (w/v) solution of whey protein concentrate that had been dialysed to remove the lactose and salts, the gel strength increased to a maximum with the addition of 0.011M $CaCl_2$. With further addition of $CaCl_2$, the gel strength decreased, possibly due to excessive cross-linking and rapid aggregation, which limits further protein unfolding and network formation (Mangino, 1992).

Treatment of whey protein concentrate with polyphosphate also improved gel strength (Nakai & Li-Chen, 1985) ostensibly because the polyphosphate chelated the excess calcium.

Johns & Ennis (1981) replaced calcium in a whey protein solution with sodium. The process was carried out by ultra-filtration. They observed that as the calcium was replaced, the resulting heat-induced gels became more translucent, more elastic, more cohesive, gummier, harder and springier.

A strong negative relationship between gel strength and calcium content was found in gels made from whey protein concentrate prepared by ultra-filtration. It was concluded that the calcium content of commercially available whey protein concentrates (0.25-1.06%) was already in the range that inhibited gel formation (Kohnhurst & Mangino 1985; Morr, 1992).

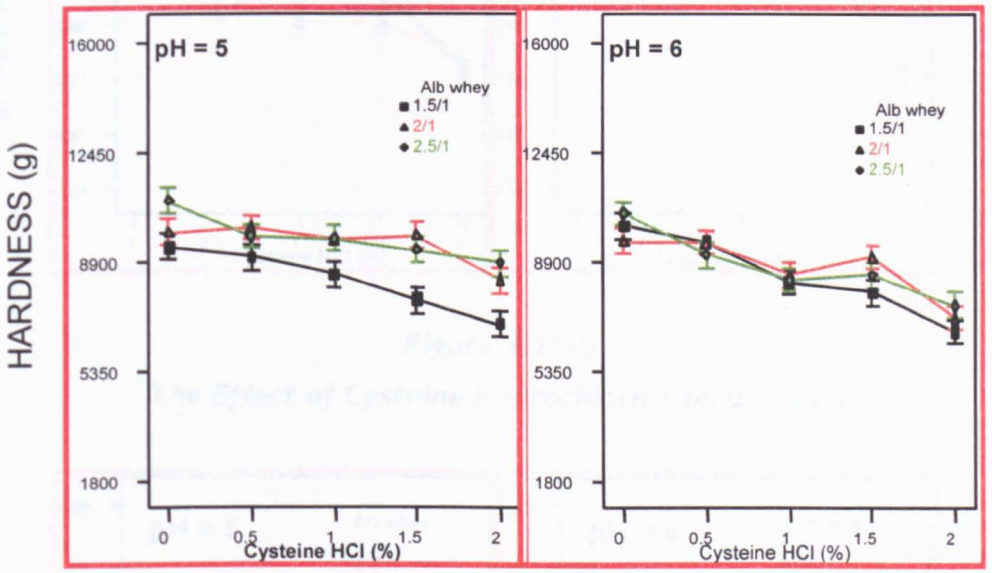
4.11 The Effect Of Cysteine Hydrochloride

Elastic Properties

Addition of cysteine hydrochloride to the gel systems had a bigger effect on gel hardness at pH 6.0 than it did at pH 5.0 as shown by the higher values of hardness with each corresponding binder type (Fig. 4.11-A). At the higher pH value, there was a progressive reduction across all samples. However at pH 5.0, only the 1.5/1 gel showed a statistically significant reduction in gel hardness. The fact that this sample had the highest proportion of whey protein concentrate suggests that the cysteine hydrochloride had a bigger effect on the whey protein than it did on the albumen.

Figure 4.11-A

The Effect of Cysteine Hydrochloride on Gel Hardness



Plastic Properties

Springiness at pH 5.0 was not affected by cysteine hydrochloride until the concentration reached about 1.5% (Fig. 4.11-B) whereas at the higher pH of 6.0, the lag in the response only holds until the concentration reached 0.5%. Further additions reduced the gel springiness to similar levels to what was observed at pH 5.0.

The *a*-value increased slightly at pH 5.0 when the concentration of the cysteine hydrochloride is raised from 0 – 1.5% (Fig. 4.11-G). A

much larger rate of increase was observed at pH 6.0 when the concentration of the cysteine hydrochloride was raised from 0.5 – 1.0% after which there was no further change. The overall conclusion is that cysteine hydrochloride can be used to reduce the gel hardness without significantly changing the elastic properties.

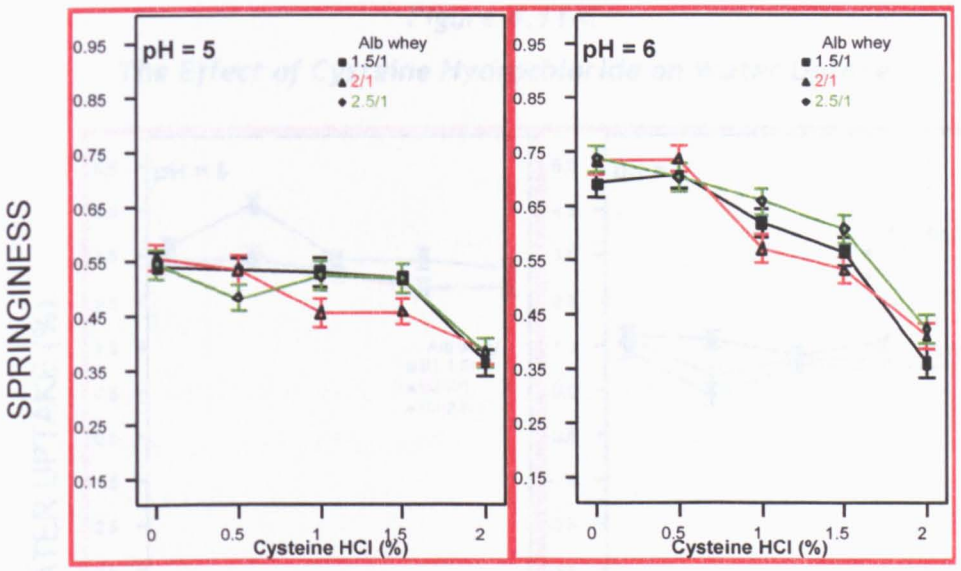
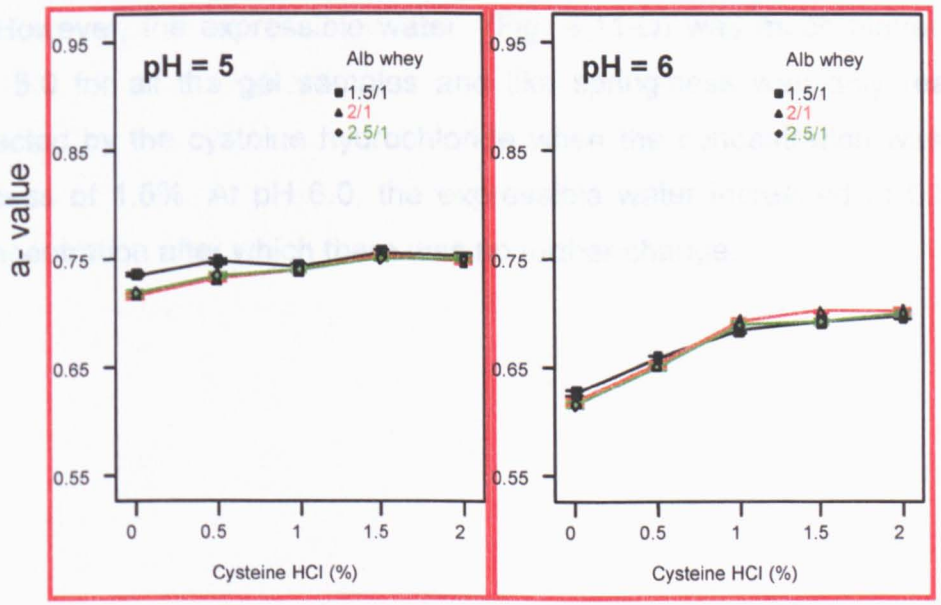


Figure 4.11-G

The Effect of Cysteine Hydrochloride on a - Value

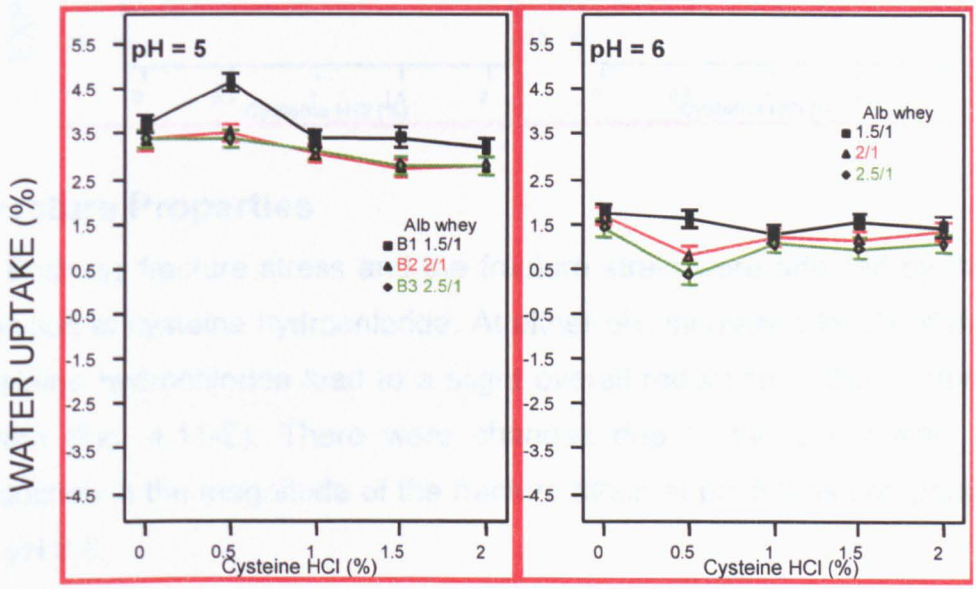


Micro-structure

Figure 4.11-C shows that the change in water uptake with the addition of cysteine hydrochloride was not very large. All the activity and differences seem to be concentrated around the 0.5% concentration level after which there was no further effect. This is especially true for the 1.5/1 gel at pH 5.0 where there was a small increase. The opposite occurred in binders 2.5/1 and 2/1 at pH 6.0 where a small reduction was observed.

Figure 4.11-C

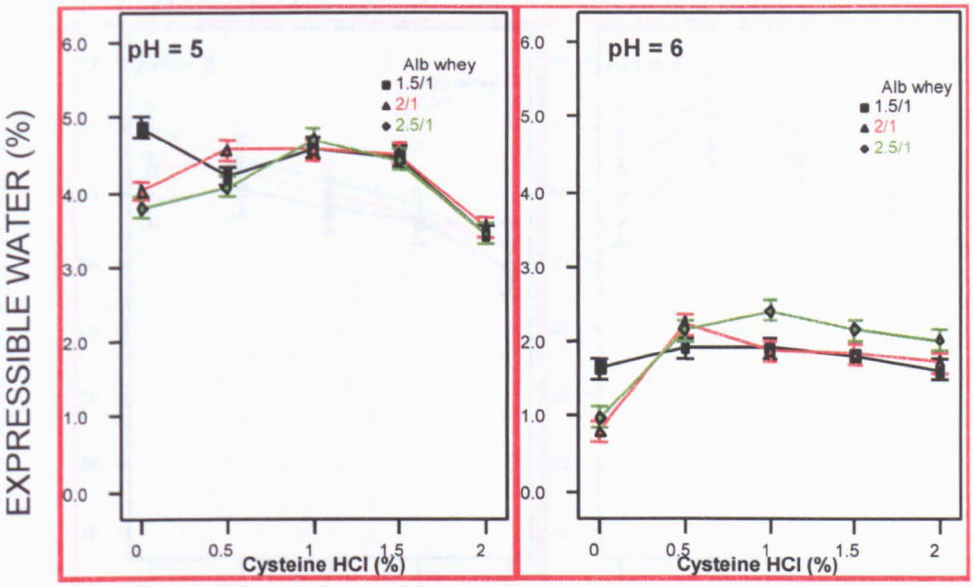
The Effect of Cysteine Hydrochloride on Water Uptake



However, the expressible water (Fig. 4.11-D) was much higher at pH 5.0 for all the gel samples and like springiness was only really affected by the cysteine hydrochloride when the concentration was in excess of 1.5%. At pH 6.0, the expressible water increased at 0.5% concentration after which there was no further change.

Figure 4.11-D

The Effect of Cysteine Hydrochloride on the Expressible Water



Fracture Properties

Both the fracture stress and the fracture strain were affected by the addition of cysteine hydrochloride. At either pH, increased levels of the cysteine hydrochloride lead to a slight overall reduction in the fracture strain (Fig. 4.11-E). There were changes due to the pH, mainly a reduction in the magnitude of the fracture strain at pH 5.0 as compared to pH 6.0.

The same is true of the fracture stress (Fig. 4.12-F), which was reduced as the concentration of the cysteine hydrochloride was increased. There was interaction between the concentration of cysteine hydrochloride and pH as shown by the overlapping lines in the graph. The overall effect of the addition of cysteine hydrochloride was not just that it softened the gels such that they were less able to resist fracturing under pressure, but that they also become more brittle.

Figure 4.11-E

The Effect of Cysteine Hydrochloride on Fracture Strain

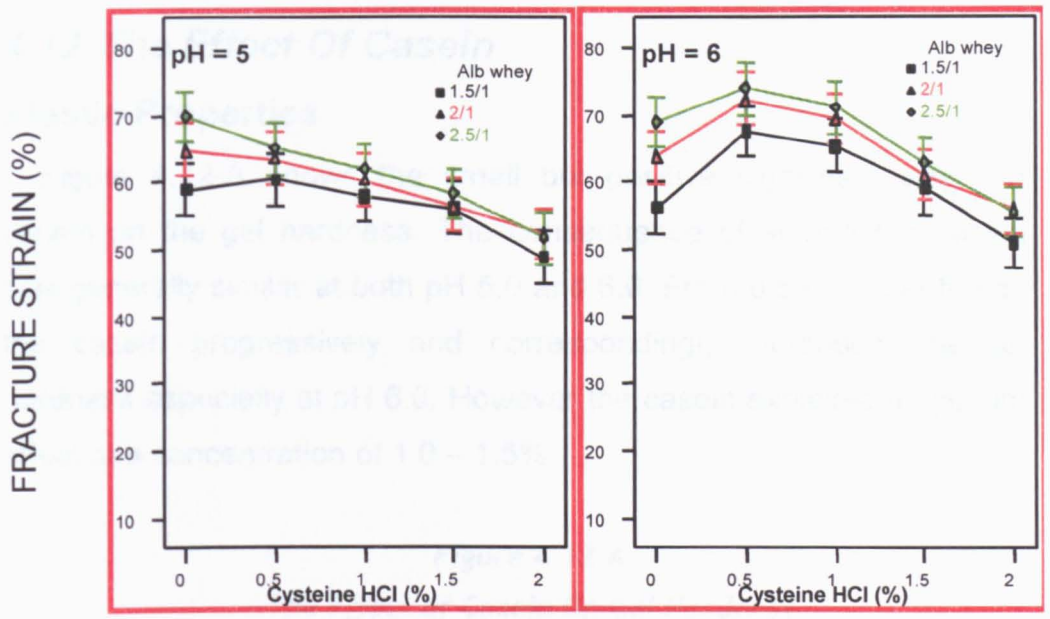
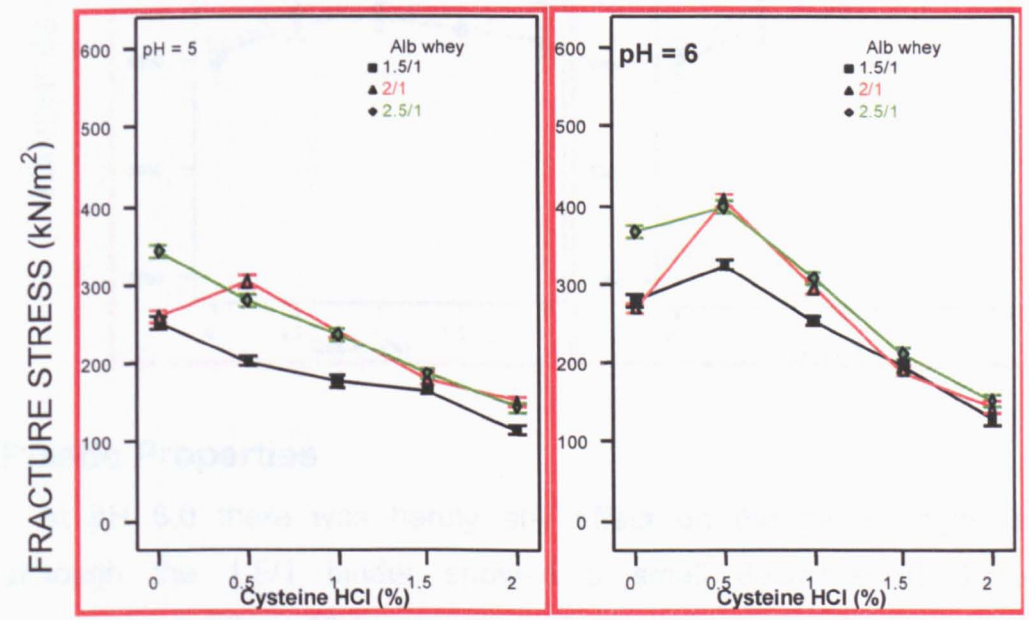


Figure 4.11-F

The Effect of Cysteine Hydrochloride on Fracture Stress



Schmidt *et al.* (1979) observed that the addition of cysteine to whey protein concentrate gel at levels up to 0.0097M increased the gel strength but at higher levels, there was a dramatic reduction in gel

strength. In addition, there was an increase in syneresis as measured by the compressible water.

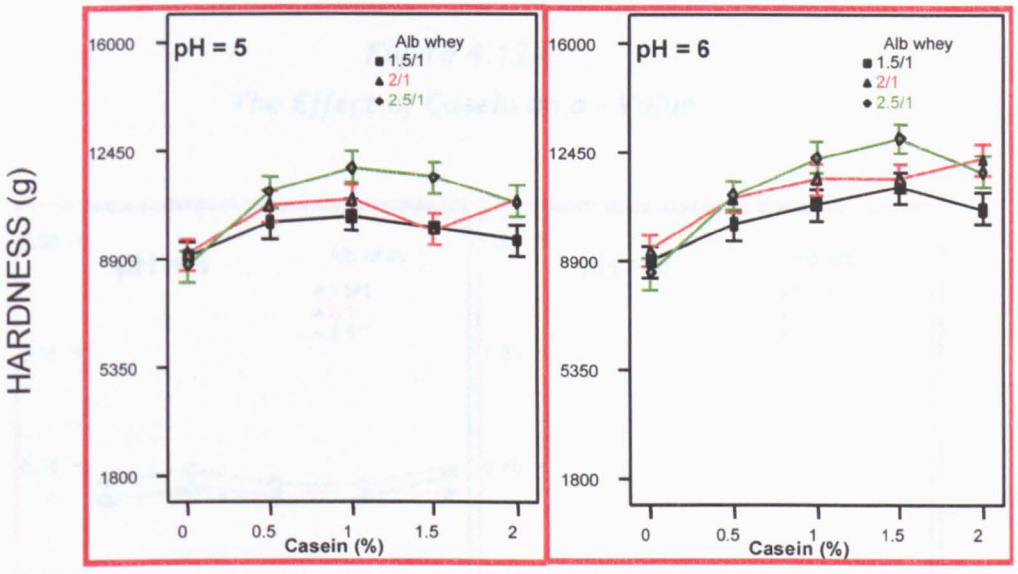
4.12 The Effect Of Casein

Elastic Properties

Figure 4.12-A shows the small but positive significant effect of casein on the gel hardness. The consequence of adding the casein was generally similar at both pH 5.0 and 6.0. From 0.5% concentration the casein progressively and correspondingly increased the gel hardness especially at pH 6.0. However the casein exhibited maximum effect at a concentration of 1.0 – 1.5%.

Figure 4.12-A

The Effect of Casein On Gel Hardness



Plastic Properties

At pH 6.0 there was hardly any effect on the gel springiness although the 1.5/1 binder showed a small decrease at 0.5% concentration (Fig. 4.12-B). But at the lower pH value of 5.0, there was a small reduction in the gel springiness when the concentration of casein was increased up to about 1.5%.

The *a*-value was higher at pH 5.0 than at pH 6.0 and the effect due to pH was greater than that of the concentration of casein (Fig 4.12-G).

Figure 4.12-B

The Effect of Casein on Gel Springiness

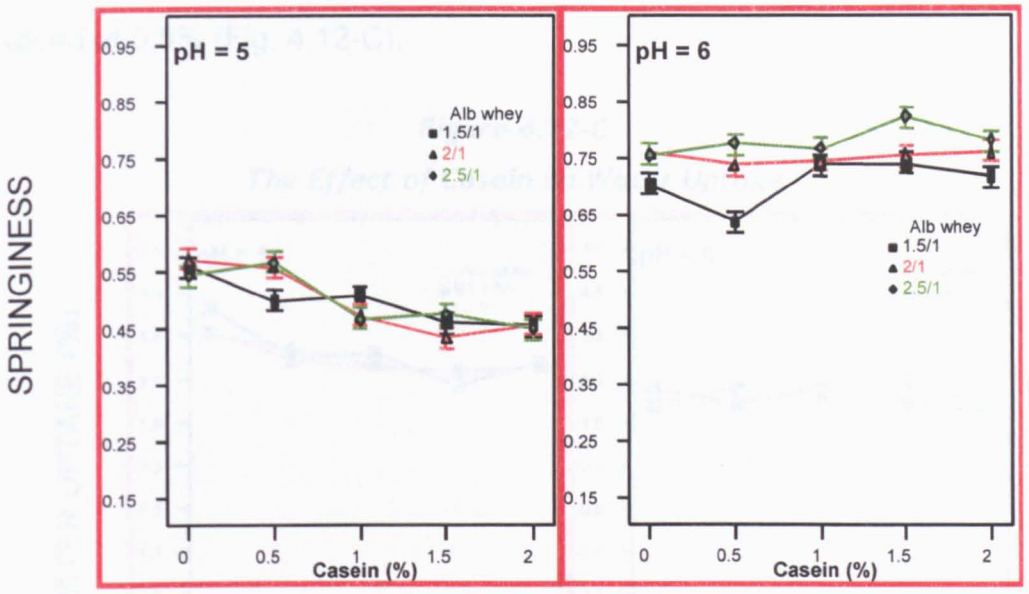
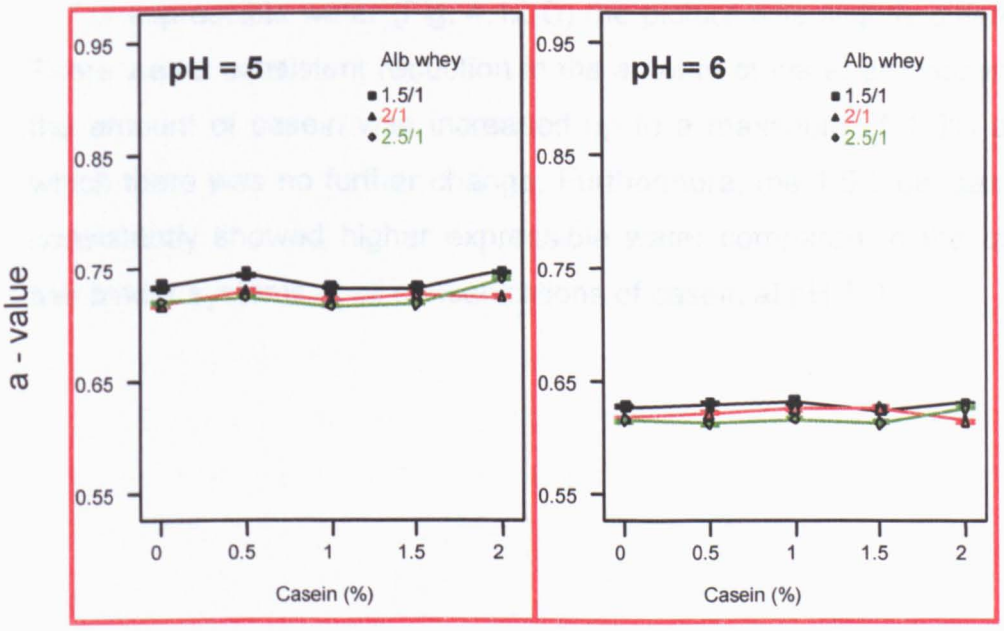


Figure 4.12-G

The Effect of Casein on a - Value

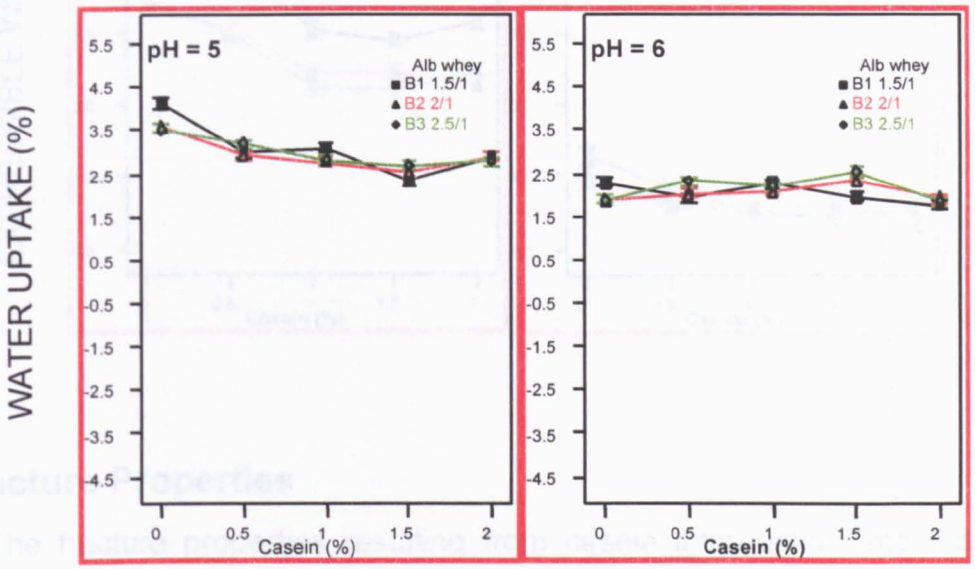


Micro-structure

Casein had no effect on the water uptake at pH 6.0. At the lower pH value, there was a slight drop in the water uptake when casein was added at 0.5% (Fig. 4.12-C).

Figure 4.12-C

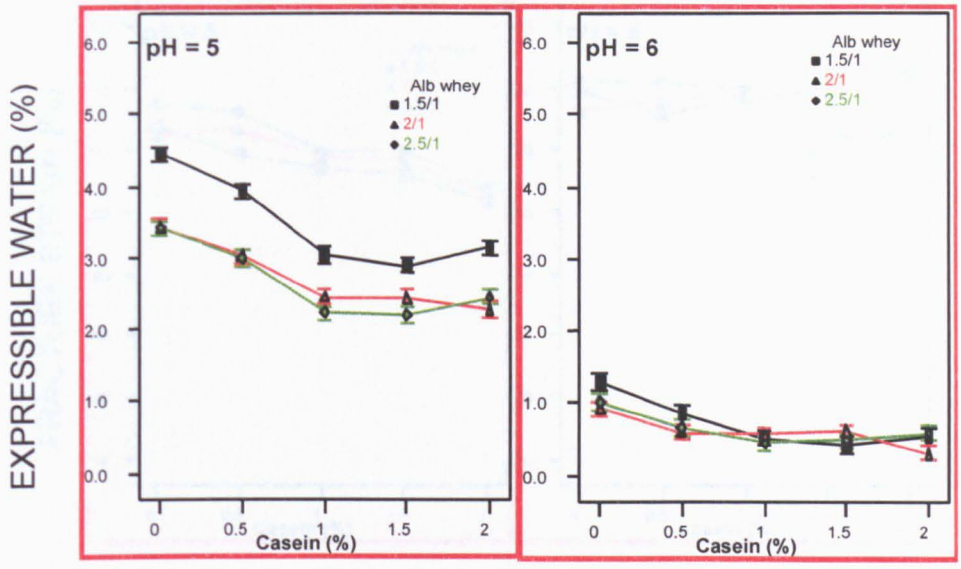
The Effect of Casein on Water Uptake



For expressible water (Fig. 4.12-D) the picture was slightly different. There was a consistent reduction in the amount of water expressed as the amount of casein was increased up to a maximum of 1.0% after which there was no further change. Furthermore, the 1.5/1 gel sample consistently showed higher expressible water compared to the other two binder systems at all concentrations of casein at pH 5.0.

Figure 4.12-D

The Effect of Casein on Expressible Water



Fracture Properties

The fracture properties resulting from casein introduction into the binder systems are represented in Figures 4.12-E and 4.12-F. Fracture strain was affected by the presence of casein and a change in the binder composition at pH 6.0 up to 2.0% addition. However at pH 5.0, there was a bigger linear reduction in the fracture strain of the gel. Increasing the amount of albumen in the binder also leads to a corresponding increase in the fracture strain.

The fracture stress was affected differently. At pH 5.0, the stress was reduced with increasing levels of casein, whereas at pH 6.0, the opposite occurs: after an initial lag, the stress at fracture increased when the concentration of casein was in excess of 1.0%. The effect of casein at the different pH values is quite an interesting one because, depending on at the pH value, casein can either increase the brittleness of the gel, or reduce it. Casein itself is not coagulated by heat but can be precipitated by acids. The closer the gel pH is to the isoelectric pH, the more the likelihood of casein precipitation, which may explain the increasing brittleness at pH 5.0.

Figure 4.12-E

The Effect of Casein on Fracture Strain

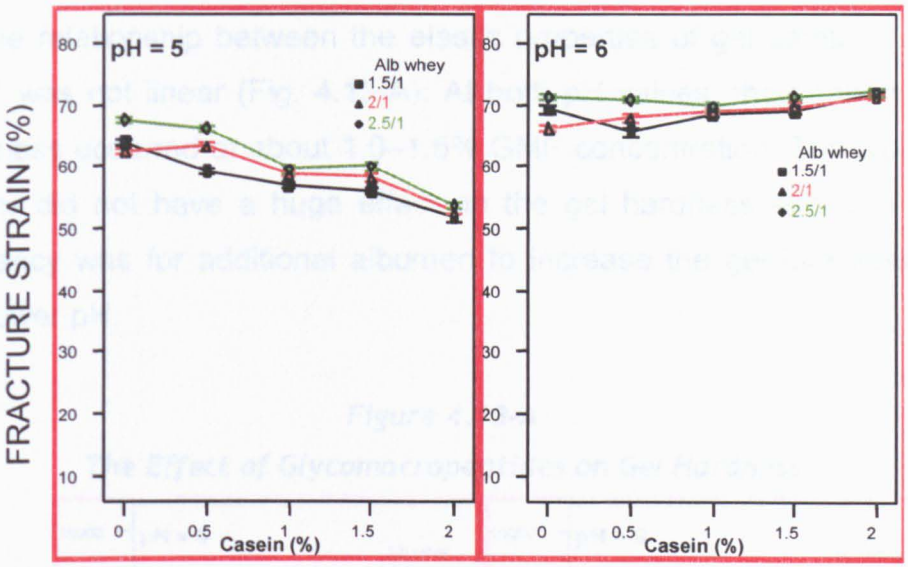
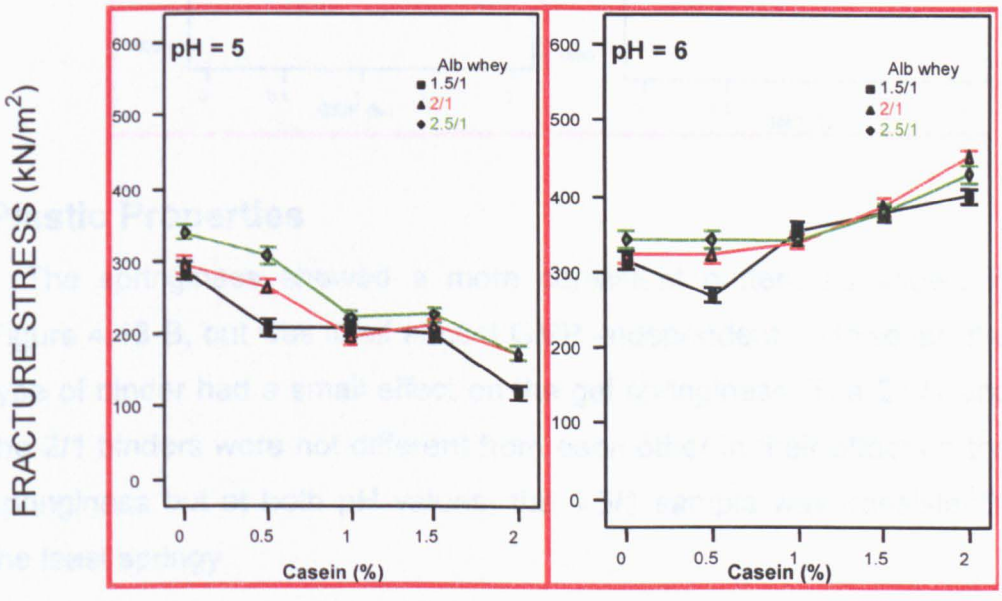


Figure 4.12-F

The Effect of Casein on Fracture Stress



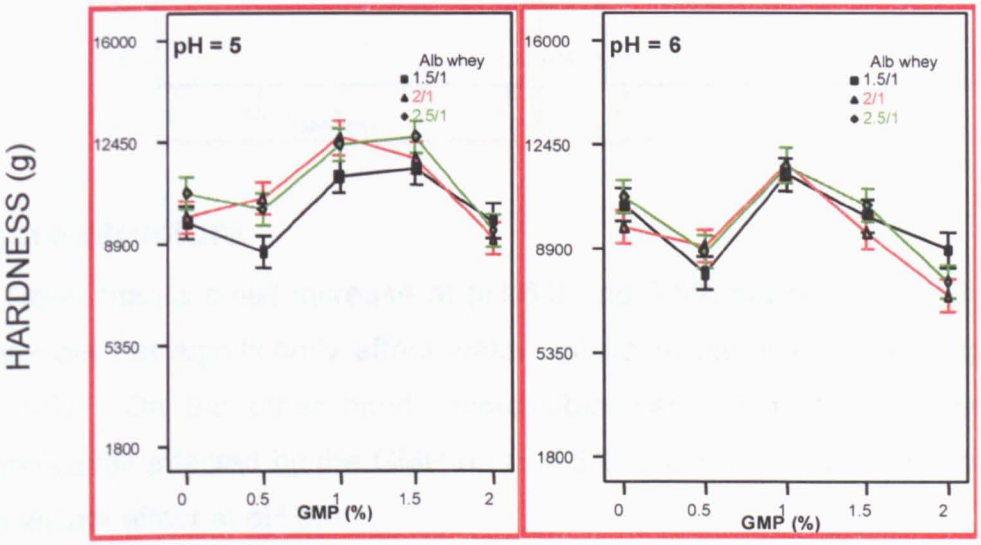
4.13 The Effect Of Glycomacropeptides (GMP)

Elastic Properties

The relationship between the elastic properties of gel samples and GMP was not linear (Fig. 4.13-A). At both pH values, the peak in gel hardness occurred at about 1.0–1.5% GMP concentration. The type of binder did not have a huge effect on the gel hardness although the tendency was for additional albumen to increase the gel hardness at the lower pH.

Figure 4.13-A

The Effect of Glycomacropeptides on Gel Hardness

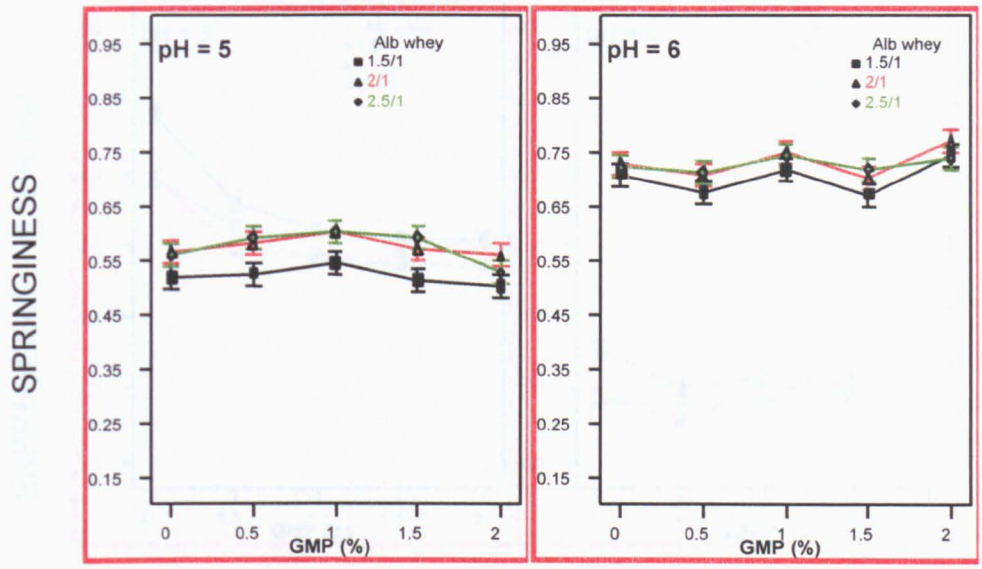


Plastic Properties

The springiness showed a more consistent pattern as shown in Figure 4.13-B, but was itself almost GMP–independent. However, the type of binder had a small effect on the gel springiness. The 2.5/1 and the 2/1 binders were not different from each other in their effect on the springiness but at both pH values, the 1.5/1 sample was consistently the least springy.

Figure 4.13-B

The Effect of Glycomacropeptides on Gel Springiness



Micro-structure

Other than a small increase at pH 6.0 and 0.5% concentration, the GMP did not significantly affect water uptake at either pH level (Fig. 4.13-C). On the other hand, expressible water (Fig. 4.13-D) was significantly affected by the GMP up to 0.5% at pH 5.0. There was no significant effect at pH 6.0.

Figure 4.13-C

The Effect of Glycomacropeptide on Water Uptake

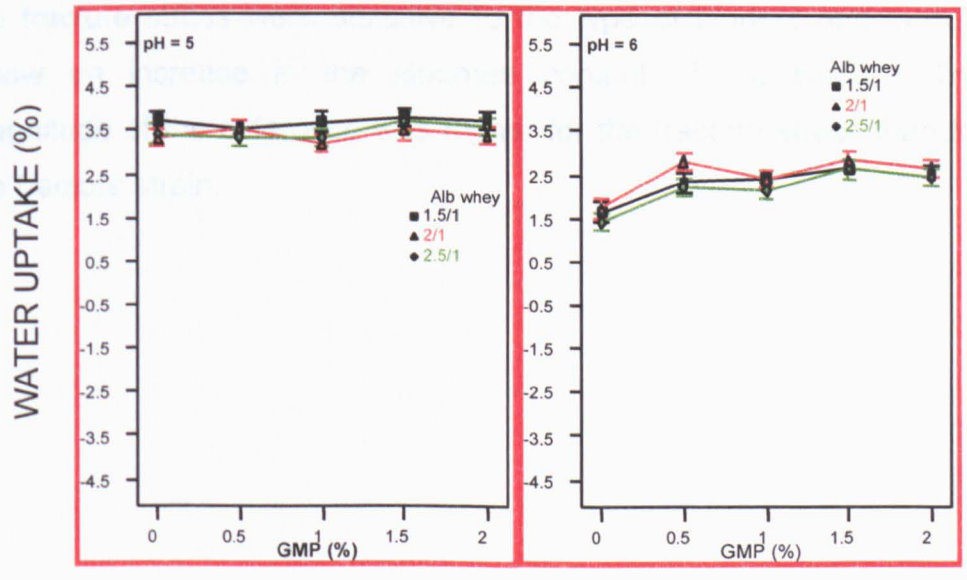
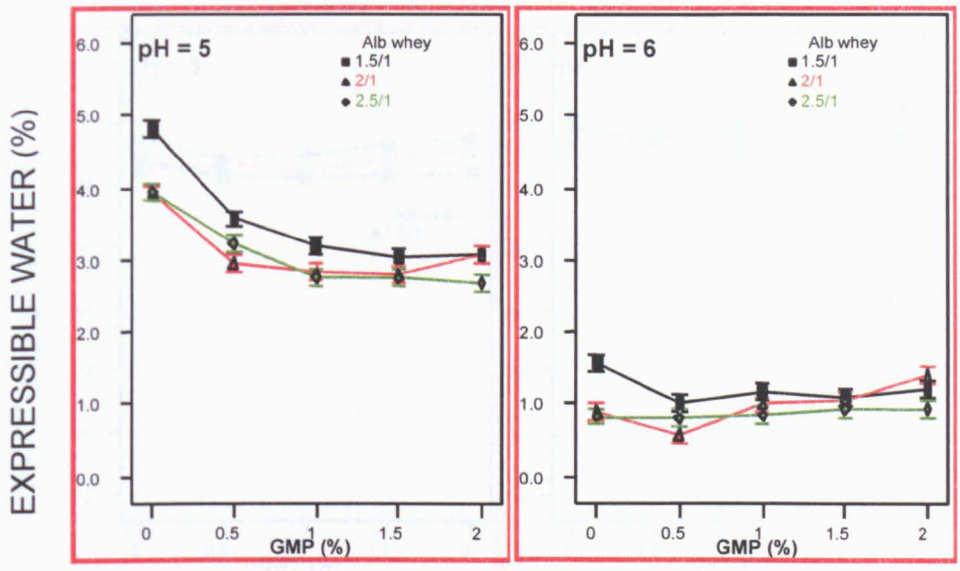


Figure 4.13-D

The Effect of Glycomacropeptides on Expressible Water



Van Vliet *et al.* (2004) reported that the effect of GMP on a heat-induced whey protein concentrate gel was to reduce the water holding capacity and strength. GMP was not incorporated into the functional network.

Fracture Properties

Both fracture properties (Figs. 4.13-E and 4.13-F) were significantly affected by altered levels of the glycomacropeptides and pH but the range of the change was small. However, both the fracture strain and the fracture stress were sensitive to the type of binders and loosely follow an increase in the albumen content of the binders. The magnitude of the difference was higher for the fracture stress than for the fracture strain.

Figure 4.14-E

The Effect of Glycomacropeptides on Fracture Strain

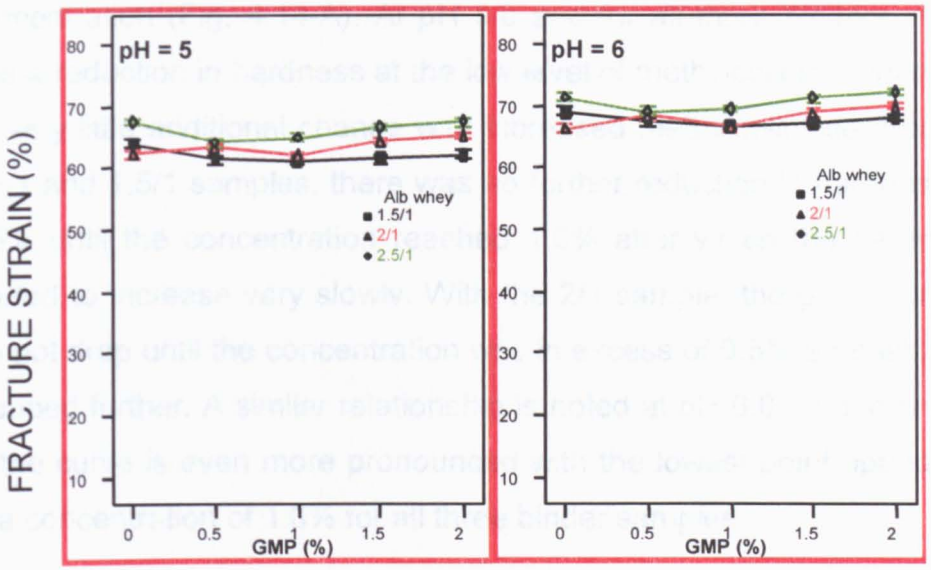
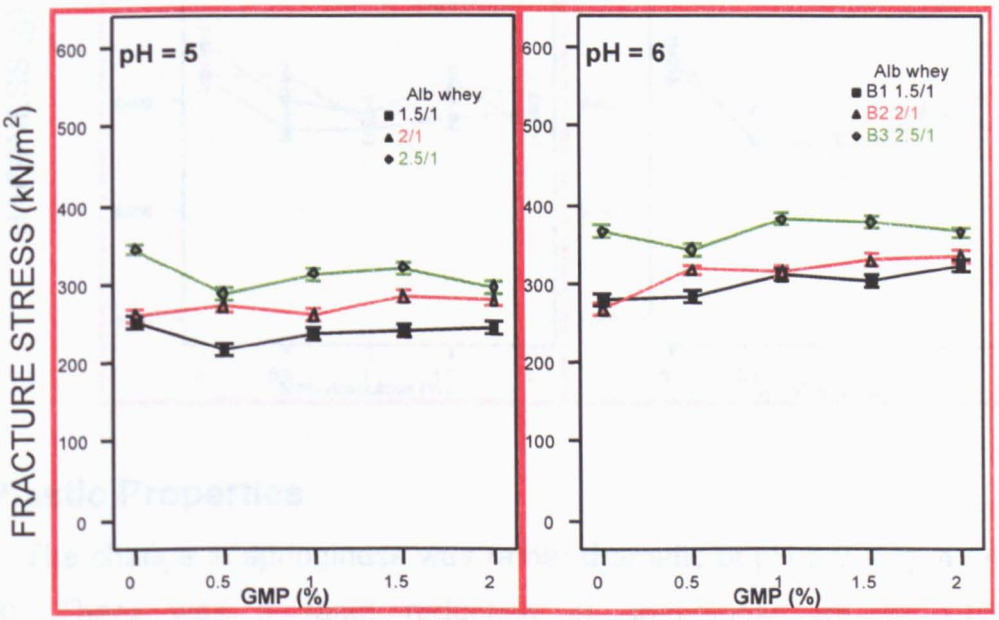


Figure 4.14-F

The Effect of Glycomacropeptides on Fracture Stress



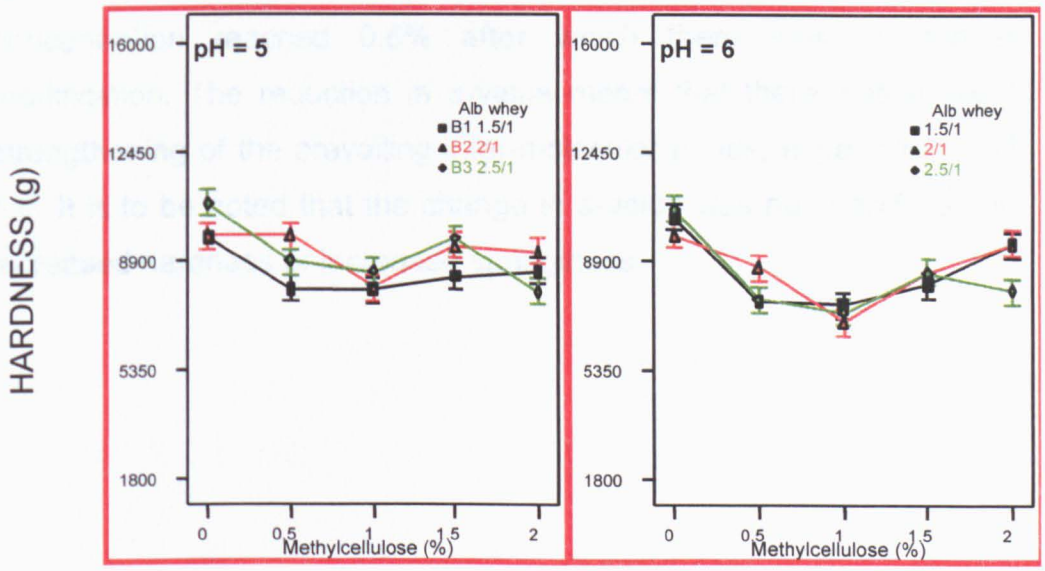
4.14 The Effect Of Methylcellulose

Elastic Properties

Methylcellulose caused a slight reduction in gel hardness up to 1.0% concentration (Fig. 4.14-A). At pH 5.0 and for all three binders, there was a reduction in hardness at the low level of methylcellulose followed by very little additional change with increased methylcellulose. For the 2.5/1 and 1.5/1 samples, there was no further reduction in hardness at 0.5% until the concentration reached 1.0% after which the hardness started to increase very slowly. With the 2/1 sample, the gel hardness did not drop until the concentration was in excess of 0.5% after which it dropped further. A similar relationship is noted at pH 6.0 but the shape of the curve is even more pronounced with the lowest point appearing at a concentration of 1.0% for all three binder samples.

Figure 4.14-A

The Effect of Methylcellulose on Gel Hardness

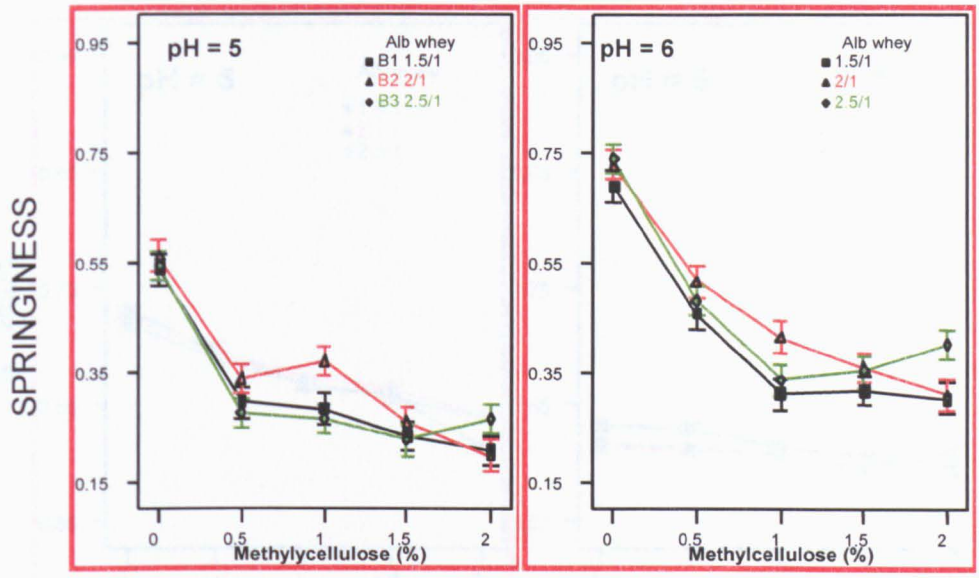


Plastic Properties

The change in springiness was rather dramatic at pH 6.0 (Fig. 4.14-B). There was a rapid reduction in springiness up to 1.0% concentration of the methylcellulose after which there was very little additional change. A similar effect was observed at pH 5.0 with the exception that the lowest point occurred at 0.5%.

Figure 4.14-B

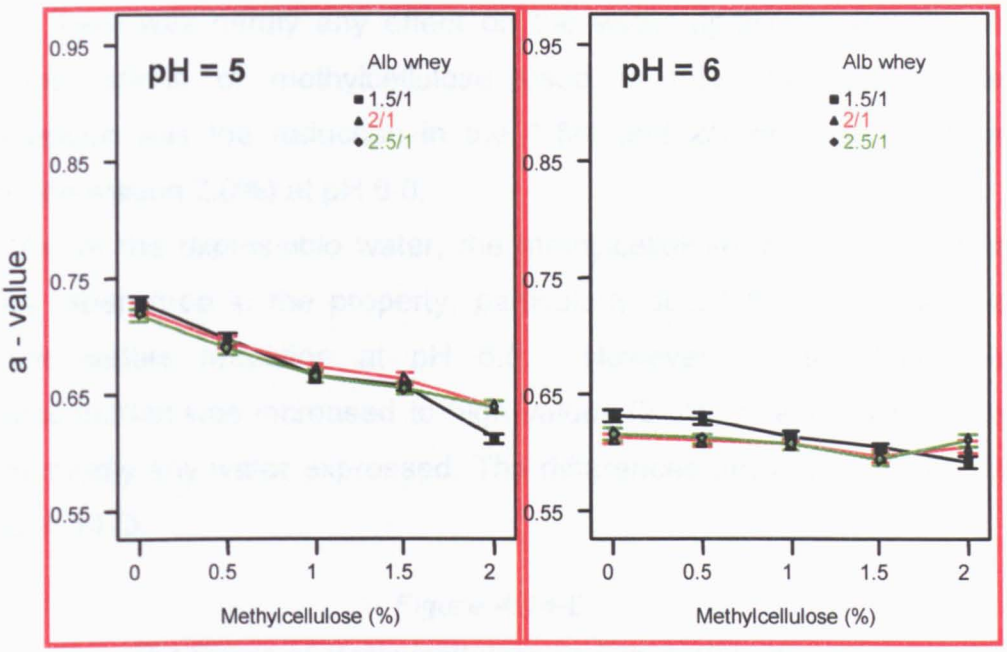
The Effect of Methylcellulose on Gel Springiness



The addition of the methylcellulose effected a reduction in the *a*-value at both pH values but the change dissipated after the concentration reached 0.5% after which there was no further modification. The reduction in *a*-value meant that there was a slight strengthening of the prevailing inter-molecular bonds, especially at pH 5.0. It is to be noted that the change in *a*-value was not manifested in increased hardness or increased springiness.

Figure 4.14-G

The Effect of Methylcellulose on a - Value

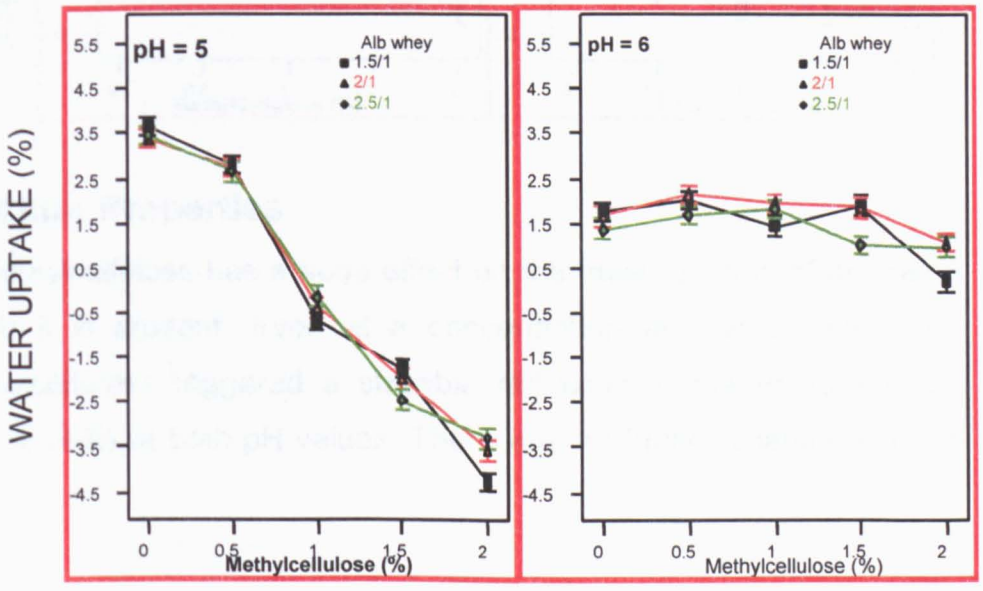


Micro-structure

Some of the biggest differences in the gel characteristics of the whole study are observed in the micro-structural properties of water uptake (Fig. 4.14-C) and expressible water (Fig. 4.14-D) for methylcellulose.

Figure 4.14-C

The Effect of Methylcellulose on Water Uptake

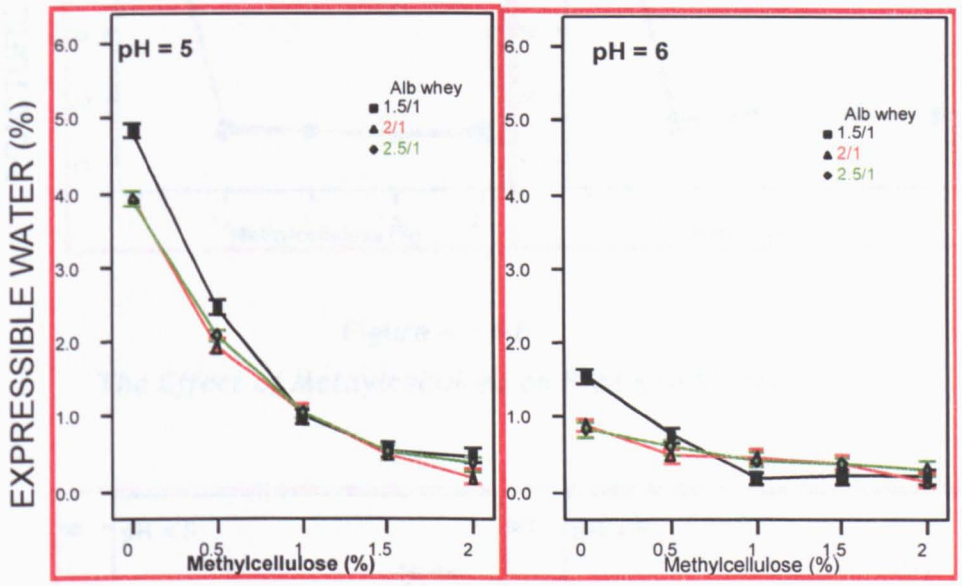


At pH 5.0, all the binder gel samples changed from exuding water (methylcellulose concentration 0.5 – 1.0%) to ones that absorbed water (methylcellulose concentration in excess of 1.0%). By contrast, at pH 6.0, there was hardly any effect on the water uptake at virtually all concentrations of methylcellulose used in this study. The sole exception was the reduction in the 1.5/1 and 2/1 gel water uptake (concentration 2.0%) at pH 6.0.

As for the expressible water, the methylcellulose increase led to a very steep drop in the property, particularly at pH 5.0. There was a more sedate reduction at pH 6.0. However, by the time the concentration was increased to high values (2.0%) at either pH, there was hardly any water expressed. The differences are shown clearly in Fig. 4.14-D.

Figure 4.14-D

The Effect of Methylcellulose on Expressible Water



Fracture Properties

Methylcellulose has a huge effect on the fracture strain of the gel in which it is present. Even at a concentration as low as 0.5%, the methylcellulose triggered a sizeable reduction in the fracture strain (Fig. 4.14-E) at both pH values. There was no further change even as

the concentration was increased further. Methylcellulose delivered one of the lowest values of fracture stress in the gel systems.

The stress at fracture (Fig.4.14-F) exhibited an unusual pattern. It was not directly affected by the pH. In addition, virtually all of the change in the fracture stress for the gel samples occurred at a concentration of methylcellulose less than 0.5%. In excess of this, there was very little further change.

Figure 4.14-E

The Effect of Methylcellulose on Fracture Strain

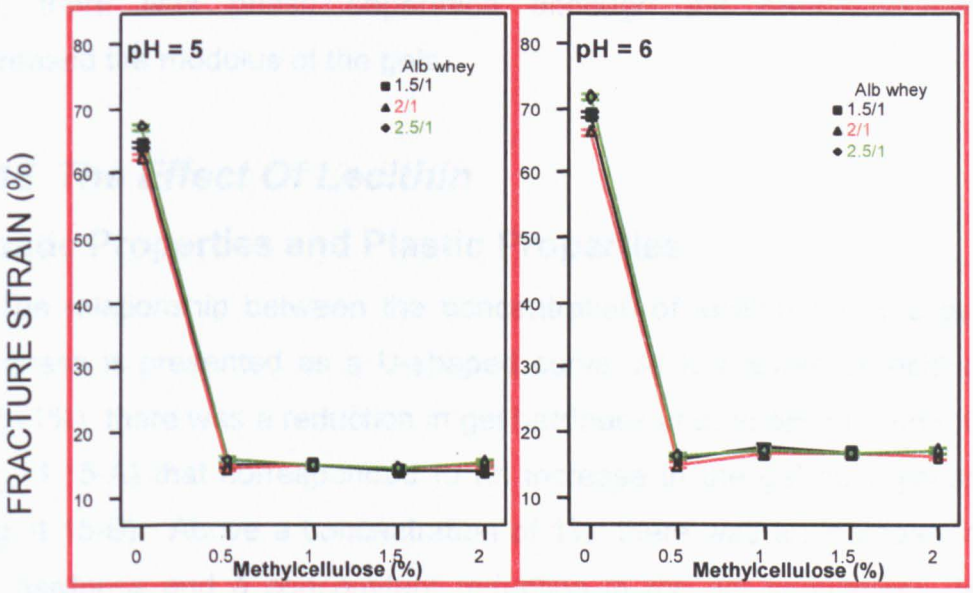
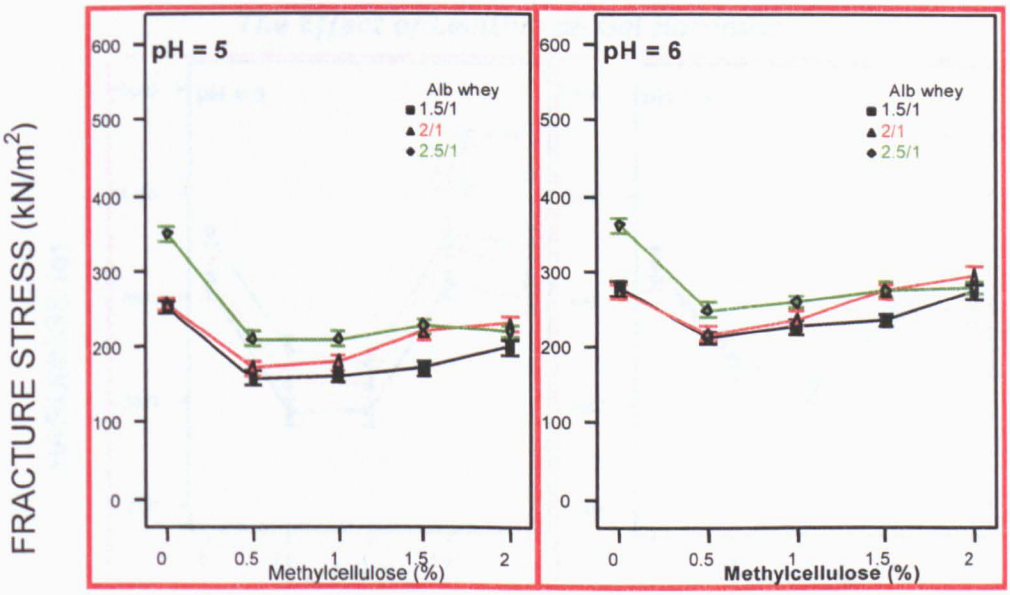


Figure 4.14-F

The Effect of Methylcellulose on Fracture Stress



The low fracture strain at concentrations of methylcellulose as low as 0.5% (Fig 4.15-E) might be indicative of phase separation and incompatibility. Turgeon & Beaulieu (2001) reported that polysaccharides such as methylcellulose show incompatibility with whey protein gels. After heating, two phases were formed at equilibrium- one that was rich in whey protein and another rich in the methylcellulose. Many such systems were characterized by fracture at low hardness.

Syrbe *et al.* (1995) proved that in a whey protein and methylcellulose gel, there was phase separation although the methylcellulose increased the modulus of the gels.

4.15 The Effect Of Lecithin

Elastic Properties and Plastic Properties

The relationship between the concentration of lecithin and the gel hardness is presented as a U-shaped curve. At low levels of lecithin (0.5–1%), there was a reduction in gel hardness at both pH 5.0 and 6.0 (Fig. 4.15-A) that corresponded to an increase in the gel springiness (Fig. 4.15-B). Above a concentration of 1%, there was an increase in gel hardness and a concomitant reduction in the gel springiness. **Is this evidence of a phase inversion or just separation because of the inability of lecithin to bind to the protein molecules?**

Figure 4.15-A

The Effect of Lecithin on Gel Hardness

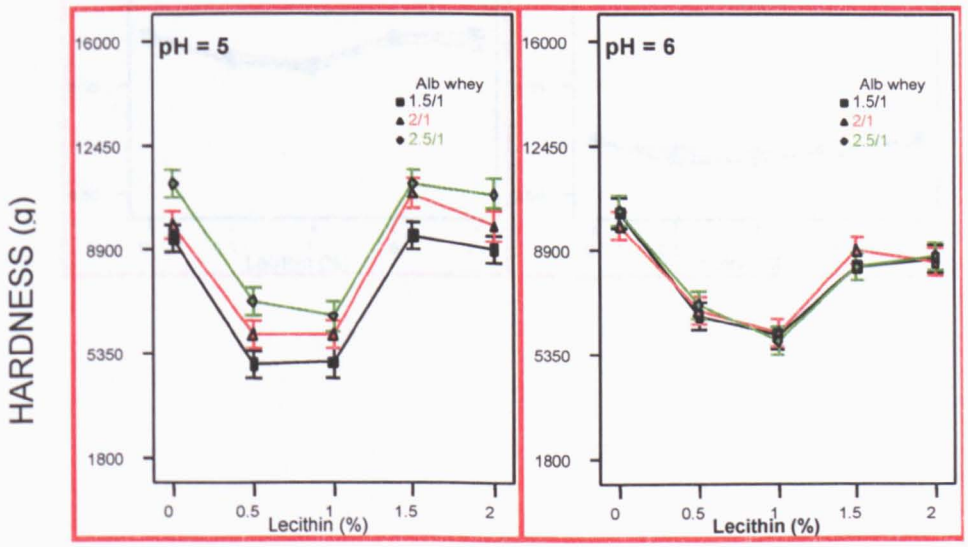
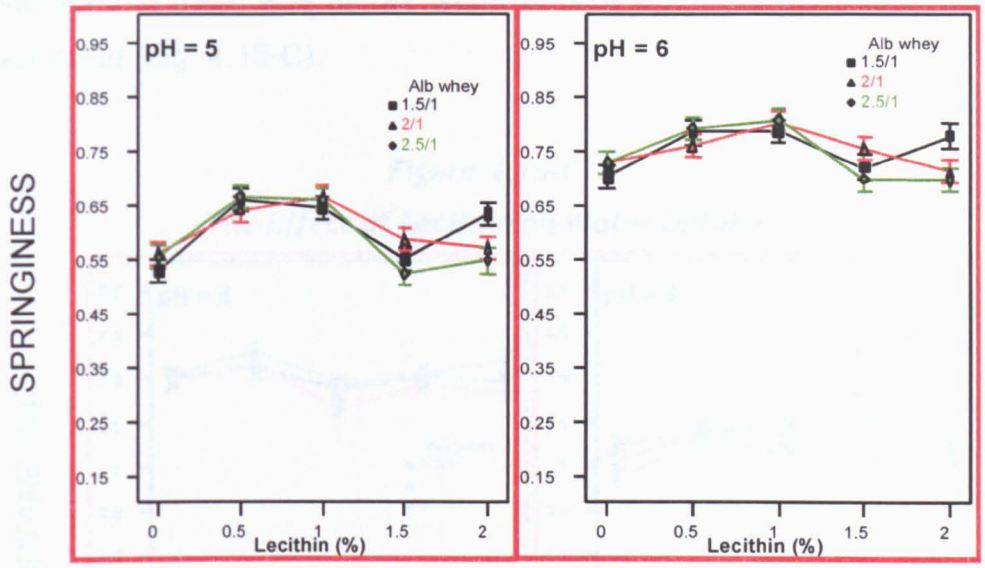
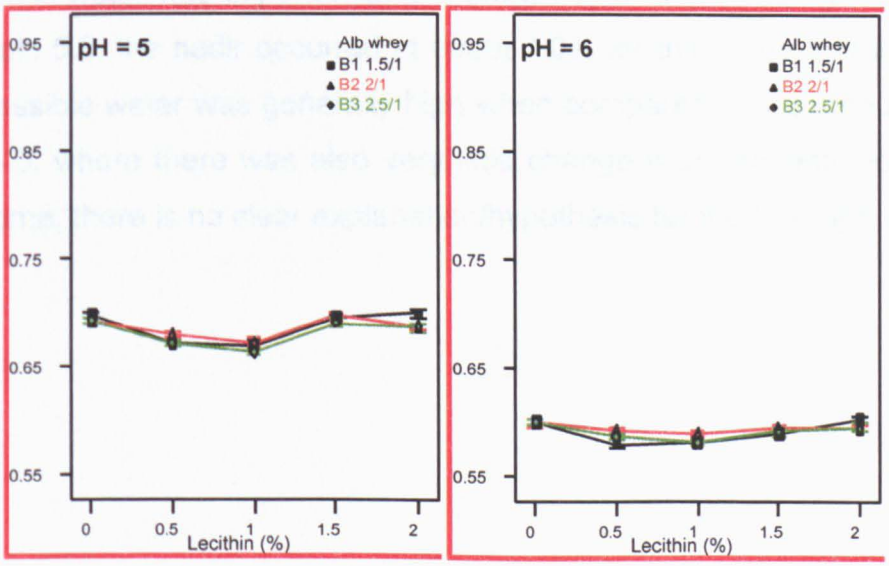


Figure 4.15-B

The Effect of Lecithin on the Gel Springiness



The plot of *a*-value followed the same pattern as the hardness and springiness (Fig 4.15-G). At pH 5.0, the lowest *a*-value occurred at lecithin concentration of 1.0%. There was no effect at pH 6.0.

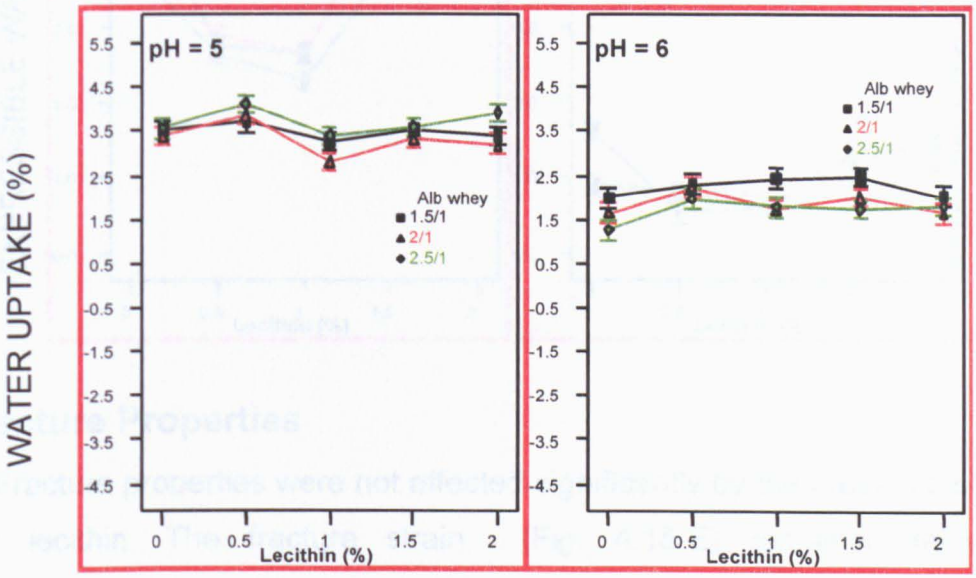


Micro-structure

The lecithin had little effect on the water uptake, although it was observed that there was higher water uptake when the pH was at the lower value (Fig. 4.15-C).

Figure 4.15-C

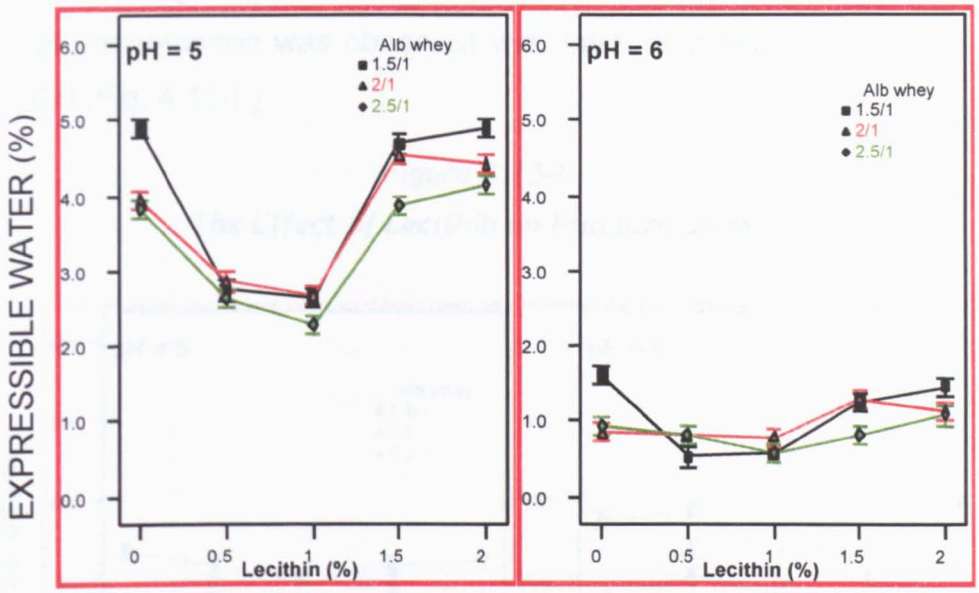
The Effect of Lecithin on Water Uptake



The relationship between the concentration of lecithin and expressible water was exponential and shown by a 'U' curve (Fig.4.15-D). At pH 5.0, the nadir occurred at about 1.0% lecithin. The quantity of expressible water was generally high when compared to the amount at pH 6.0, where there was also very little change with concentration. At this time, there is no clear explanation/hypothesis for the U shape.

Figure 4.15-D

The Effect of Lecithin on Expressible Moisture

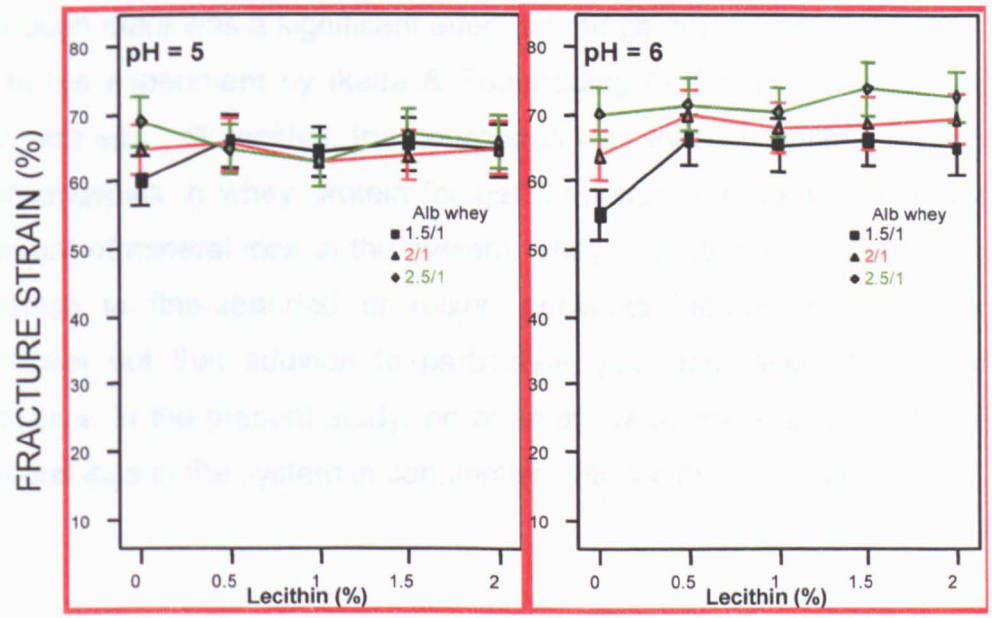


Fracture Properties

Fracture properties were not affected significantly by the presence of the lecithin. The fracture strain (Fig. 4.15-E) remained fairly independent of lecithin concentration although the larger than average error bars in the graph may indicate an experimental problem.

Figure 4.15-E

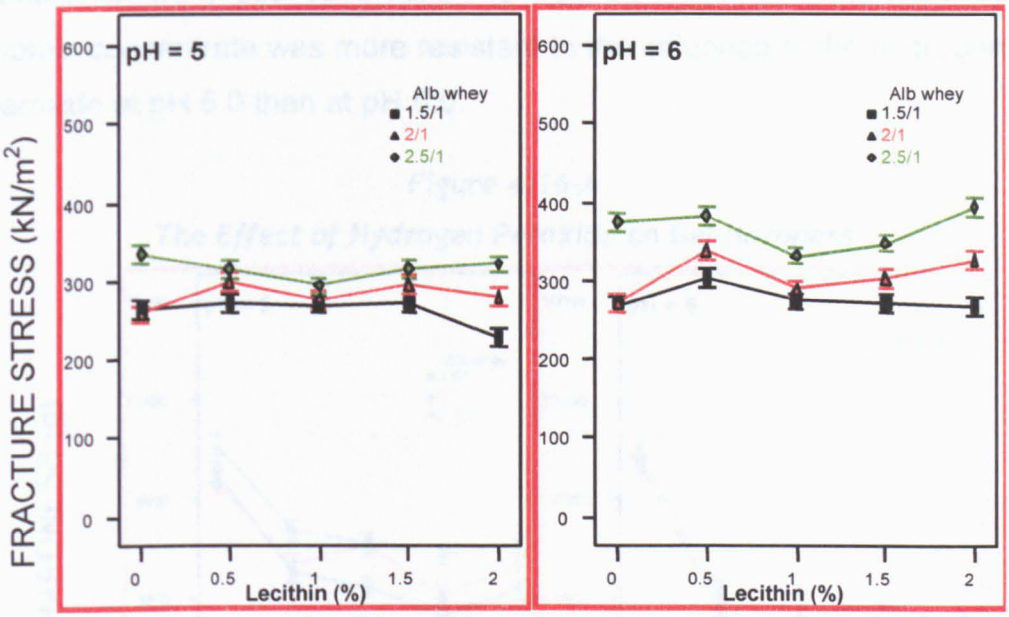
The Effect of Lecithin on Fracture Strain



At pH 6.0 the binder type had an effect on the fracture strain. The strain at fracture mimicked the increase in the albumen content of the binder showing that albumen increased the brittleness of the gel. A similar phenomenon was observed with fracture stress at both pH 5.0 and 6.0 (Fig. 4.15-F).

Figure 4.15-F

The Effect of Lecithin on Fracture Stress



Under the conditions of the present series of experiments, there was no effect of the lecithin concentration on the fracture properties although there was a significant effect on the gel hardness.

In the experiment by Ikeda & Foegedding (1999) that was carried out with egg yolk lecithin, the conclusion was that the optimal level of phospholipids in whey protein for gel formation is determined by the amount of mineral ions in the system. They had observed that lecithin addition to fine-stranded or mixed networks increased the elastic modulus but that addition to particulate gels decreased the elastic modulus. In the present study, no attempts were made to quantify the mineral ions in the system in conjunction with lecithin concentration.

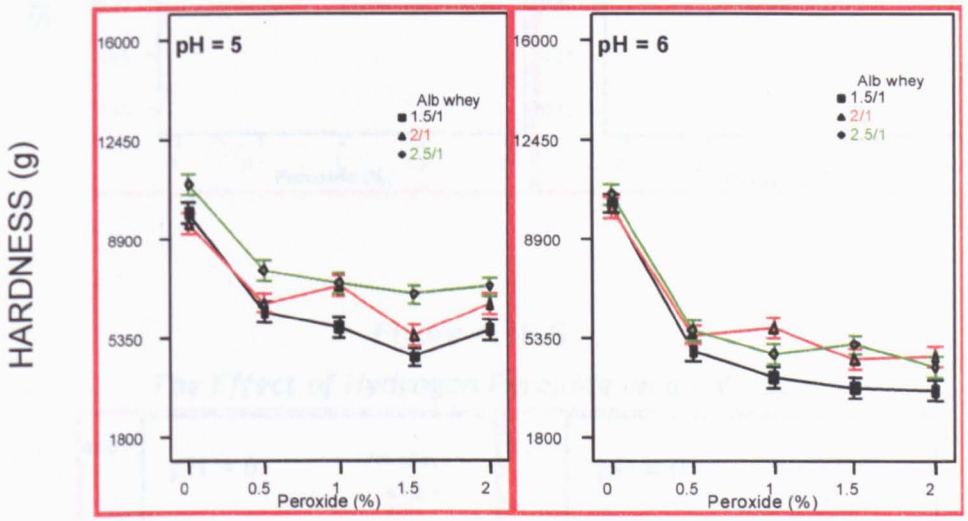
4.16 The Effect Of Hydrogen Peroxide

Elastic Properties

Hydrogen peroxide addition to the gel system had the effect of producing a rather soft gel at concentrations as low as 0.5% (Fig. 4.16-A). The rapid reduction in gel hardness affected all binder samples but the change was more pronounced at pH 6.0. The significant difference between the 2.5/1 and 2/1 gel at pH 5.0 disappeared at pH 6.0. The conclusion from this observation is that the albumen and or whey protein concentrate was more resistant to the influence of the hydrogen peroxide at pH 5.0 than at pH 6.0.

Figure 4.16-A

The Effect of Hydrogen Peroxide on Gel Hardness



Plastic Properties

Springiness was not hugely affected at pH 5.0, but even at pH 6.0, there was no change until the concentration was in excess of 1.0%. At this point, there was a massive drop in springiness.

Figure 4.16-G showed a steady increase in the *a*-value for all three binder samples at both pH values but especially at pH 6.0. The increase means that the molecular bonds became weaker as the concentration of the peroxide was raised. Noteworthy is the observation that at both pH values, there was a sharp rise in the *a*-value as the concentration was raised from 1.5% to 2% in the gel

produced from the 1.5/1 binder. The molecular bonds in the higher albumen gel samples were more resistant to the effects of the hydrogen peroxide.

Figure 4.16-B

The Effect of Hydrogen Peroxide on Gel Springiness

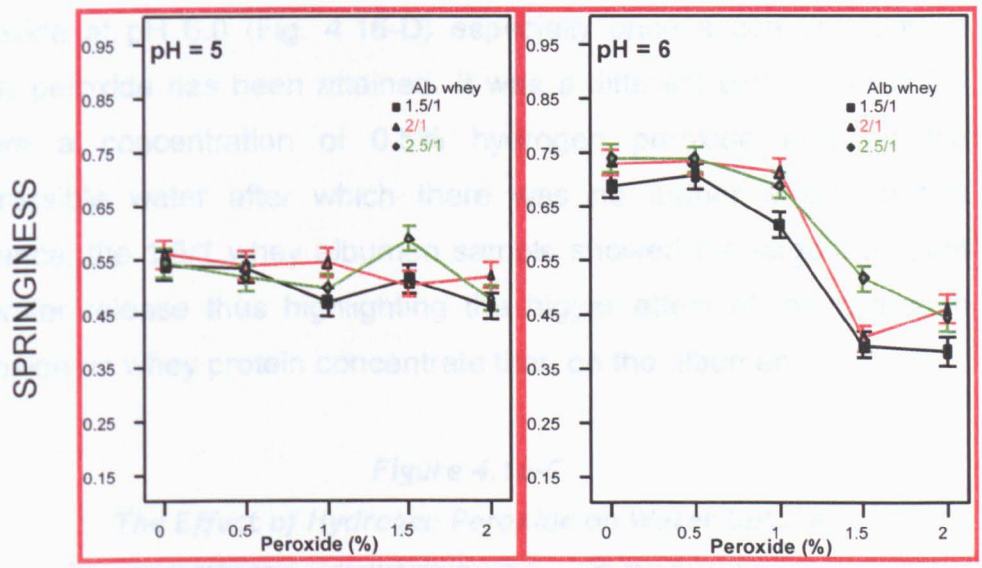
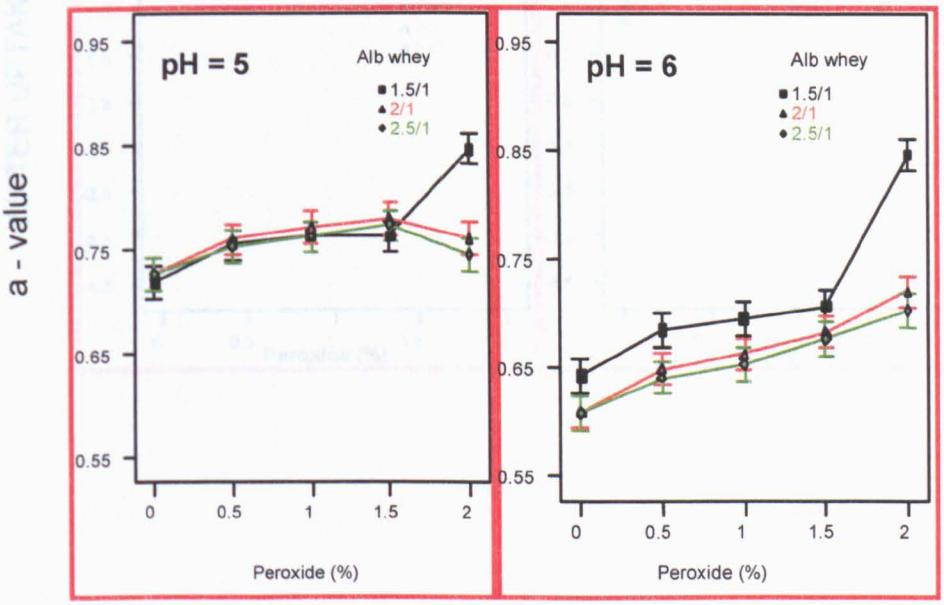


Figure 4.16-G

The Effect of Hydrogen Peroxide on a - Value



Micro-structure

Water uptake did not seem to be affected much either by the concentration of peroxide or the binder type (Fig. 4.16-C) although there was a separation between the plots of the binder samples as the concentration of hydrogen peroxide reached 2.0%.

Nor was the expressible water affected by the concentration of the peroxide at pH 6.0 (Fig. 4.16-D) especially once a concentration of 0.5% peroxide has been attained. It was a different picture at pH 5.0 where a concentration of 0.5% hydrogen peroxide reduced the expressible water after which there was no further effect. In this instance, the 1.5/1 whey albumen sample showed the largest amount of water release thus highlighting the bigger effect of the hydrogen peroxide on whey protein concentrate than on the albumen.

Figure 4.16-C

The Effect of Hydrogen Peroxide on Water Uptake

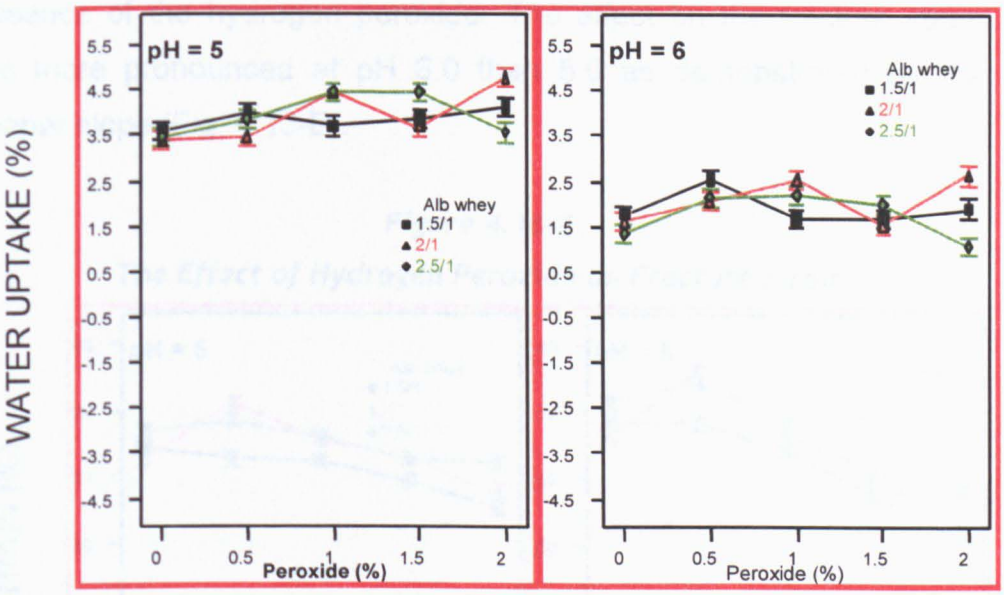
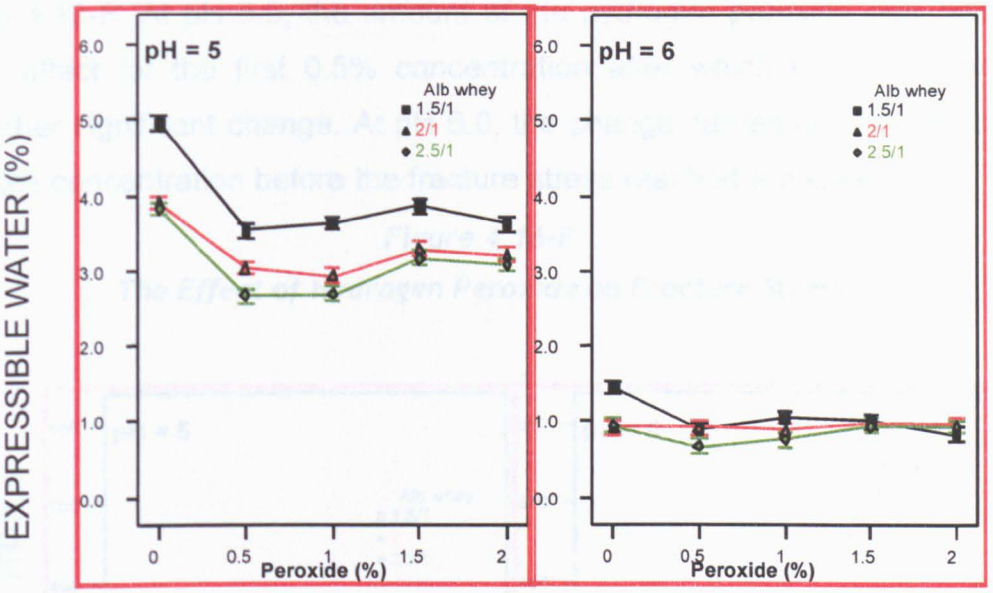


Figure 4.16-D

The Effect of Hydrogen Peroxide on Expressible Moisture

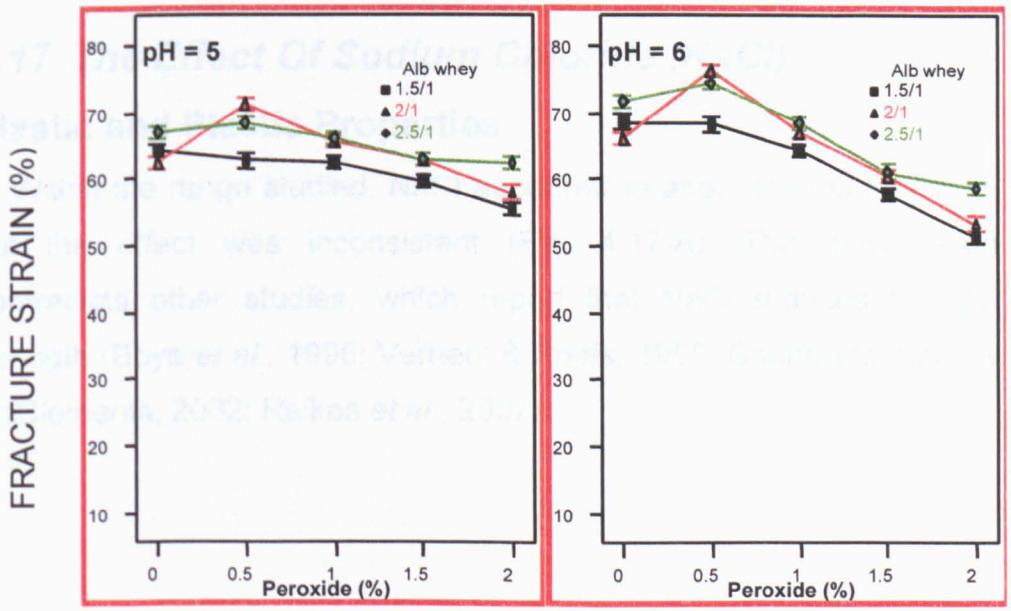


Fracture Properties

Both fracture strain and fracture stress were affected by the presence of the hydrogen peroxide. The effect on the fracture strain was more pronounced at pH 6.0 than 5.0 as demonstrated by the steeper slope (Fig. 4.16-E).

Figure 4.16-E

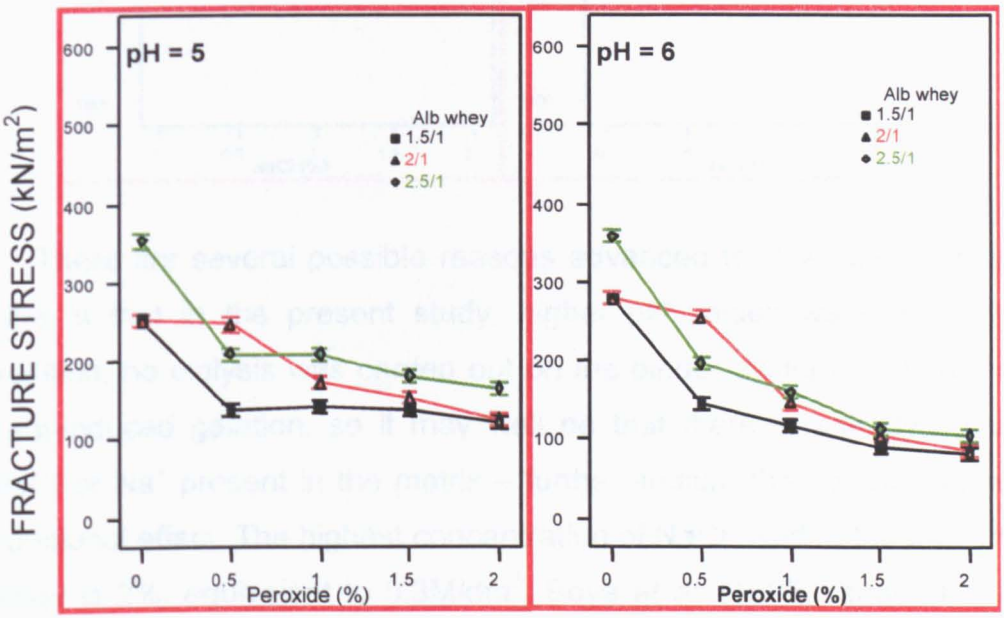
The Effect of Hydrogen Peroxide on Fracture Strain



In the case of the fracture stress, the pH generally had only a small effect as demonstrated by the almost identical profile of the graphs in Fig 4.16-F. At pH 5.0, the amount of the hydrogen peroxide only had an effect for the first 0.5% concentration after which there was no further significant change. At pH 6.0, the change carries on until about 1.5% concentration before the fracture stress reached a plateau.

Figure 4.16-F

The Effect of Hydrogen Peroxide on Fracture Stress



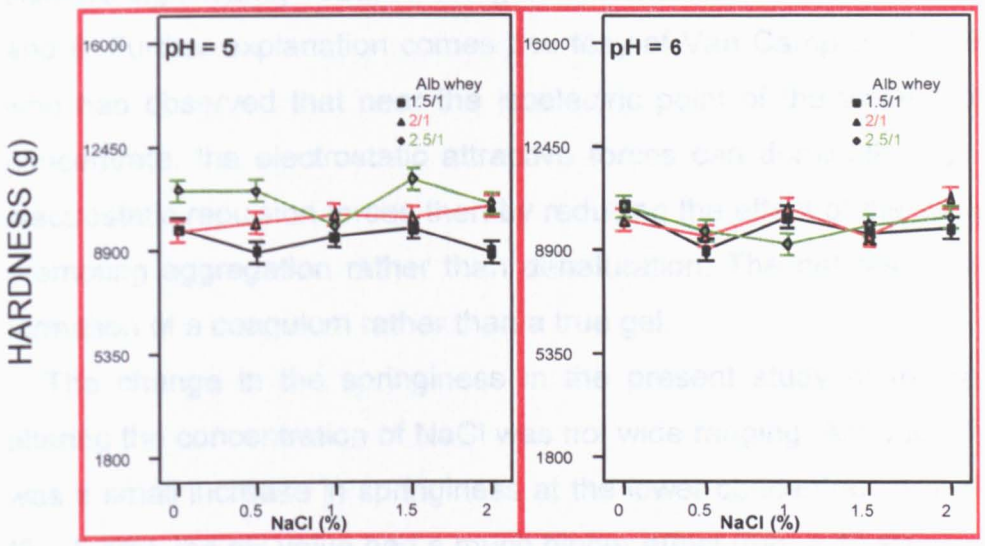
4.17 The Effect Of Sodium Chloride (NaCl)

Elastic and Plastic Properties

Within the range studied, NaCl appeared to affect the gel hardness but the effect was inconsistent (Fig. 4.17-A). This observation contradicts other studies, which report that NaCl reduces the gel strength (Boye *et al.*, 1995; Verheul & Roefs, 1998; Chantrapornchai & McClements, 2002; Raikos *et al.*, 2007).

Figure 4.17-A

The Effect of NaCl on Gel Hardness



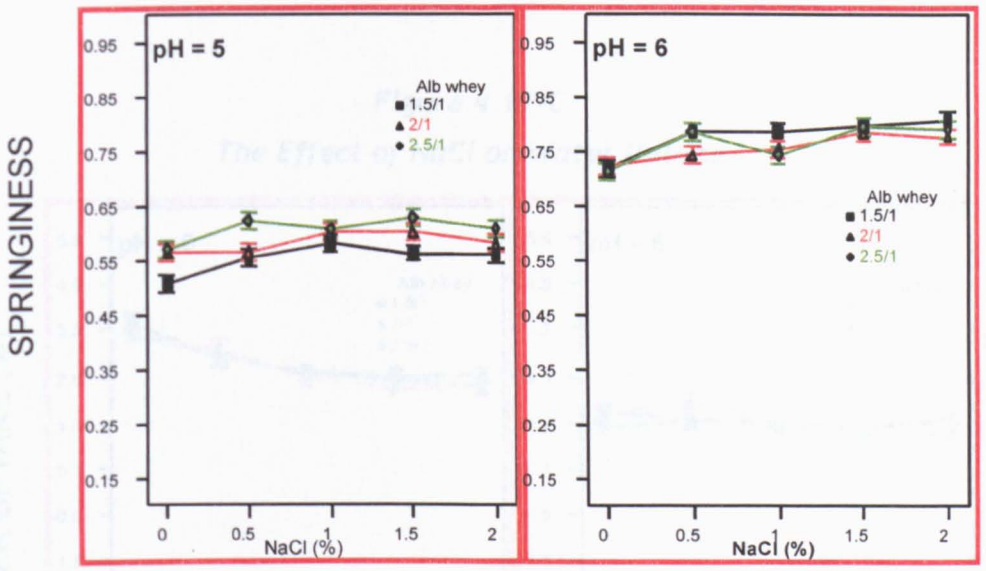
There are several possible reasons advanced for the discrepancy. One is that in the present study, higher pH values were used. In addition, no dialysis was carried out on the binder systems before the heat-induced gelation, so it may well be that there is already some NaCl or Na^+ present in the matrix – further sodium thereby having no additional effect. The highest concentration of NaCl used in the present study is 2%, equivalent to 0.3M/dm^3. Boye *et al.* (1995) reported that there was considerable variability in the gelling ability over a NaCl range of 0–2M. Firm gels were obtained in the absence of NaCl and soft gels prevailed when the NaCl was below 1M. Boye *et al.* did not state whether there was dialysis of the whey protein solution to remove any residual sodium ions. In a separate study by Verheul & Roefs (1998), the concentration of NaCl studied was in the range of 0.1-3.0 M/dm^3 but at neutral pH. It was reported that there was a marked decrease in the elastic modulus of the resulting whey protein isolate gels. In yet another study, it was shown that the gel characteristics of water loss and elastic recoverability reached a plateau when the NaCl concentration was in excess of 0.2M (Chantrapornchai & McClements, 2002).

Raikos *et al.* (2007) reported on a somewhat similar study that was carried out with egg white gels in which the addition of 6% (1M) sodium chloride significantly reduced the gel hardness at all pH values of 2, 5 and 8. Further explanation comes courtesy of Van Camp *et al.* (1997) who had observed that near the isoelectric point of the whey protein concentrate, the electrostatic attractive forces can dominate over the electrostatic repulsion forces thereby reducing the effect of the salt and promoting aggregation rather than denaturation. The net result is the formation of a coagulum rather than a true gel.

The change in the springiness in the present study observed by altering the concentration of NaCl was not wide ranging. Although there was a small increase in springiness at the lower concentration of NaCl (0 – 0.5%), the pH value had a much bigger effect (Fig. 4.17-B).

Figure 4.17-B

The Effect of NaCl on Gel Springiness



At pH 5.0, the *a*-value either stayed constant or was reduced slightly with 0.5 – 1.0% NaCl for all samples, after which there was no further change. However, at pH 6.0, there was a small increase in the *a*-value at 0.5% NaCl. Above this concentration, there was no further change. As usual, the *a*-value was higher at pH 5.0 than 6.0 showing the effect of pH on the inter-molecular bonds. The lower *a*-value for the high

albumen gel systems showed that the molecular bonds in the albumen were less susceptible to the effects of the NaCl.

Figure 4.17-G

The Effect of NaCl on a - Value

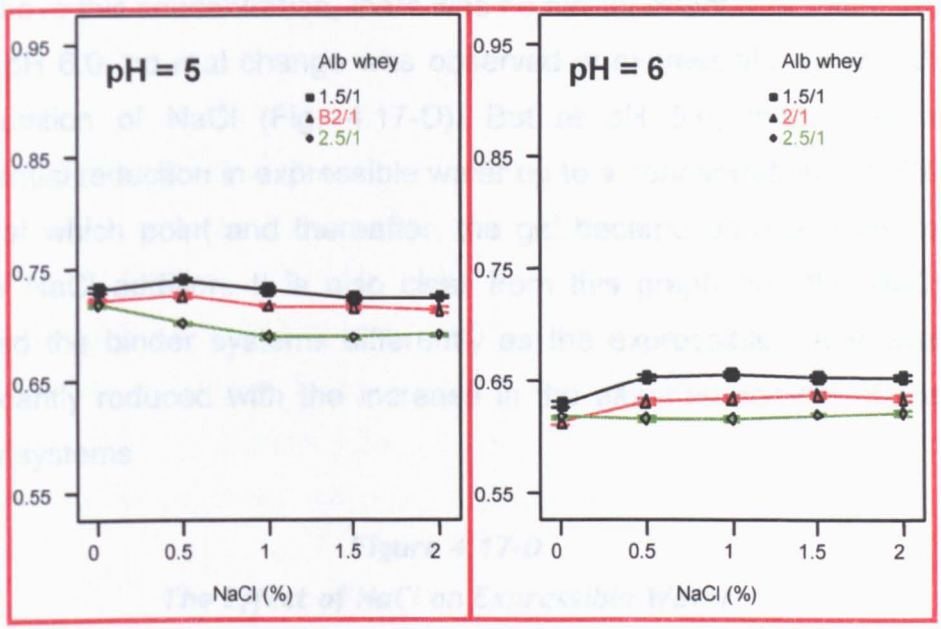
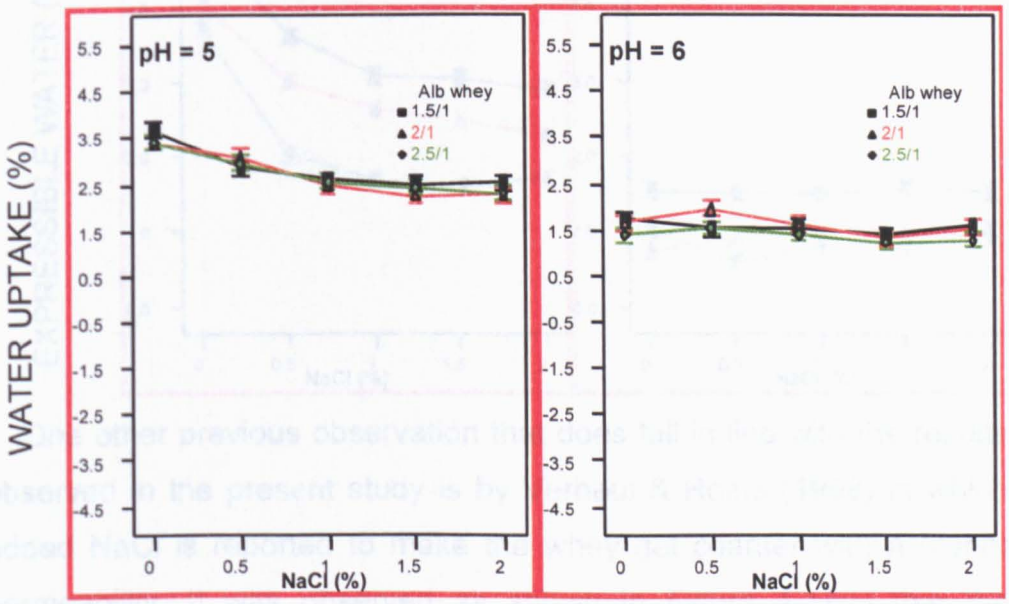


Figure 4.17-C

The Effect of NaCl on Water Uptake



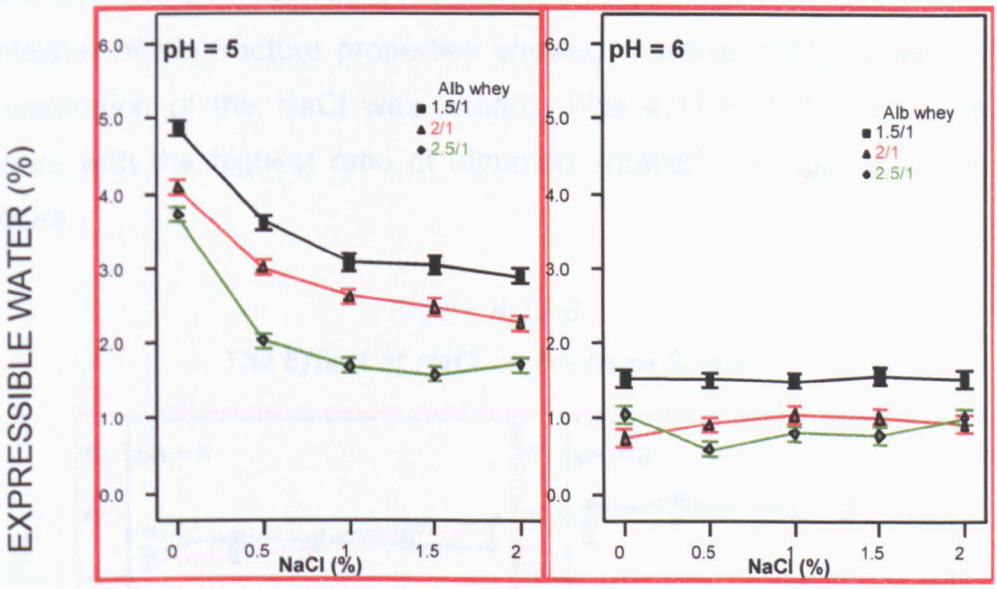
Micro-structure

At pH 6.0, the NaCl had little effect on the water uptake of any of the gel samples (Fig. 4.17-C). There was a very small reduction in the water uptake with increasing concentration of NaCl (up to 1.0%) at pH 5.0. Above this concentration, there was no further effect.

At pH 6.0, no real change was observed in expressible water with the addition of NaCl (Fig. 4.17-D). But at pH 5.0, there was a substantial reduction in expressible water up to a concentration of 0.5% NaCl at which point and thereafter, the gel became unresponsive to further NaCl addition. It is also clear from this graph that the NaCl affected the binder systems differently as the expressible water was significantly reduced with the increase in the albumen content of the binder systems.

Figure 4.17-D

The Effect of NaCl on Expressible Water



One other previous observation that does fall in line with the results observed in the present study is by Verheul & Roefs (1998) in which added NaCl is reported to make the whey gel coarser with a higher permeability. It was observed as shown in Figure 4.17-D that the expressible water changed significantly with the introduction of NaCl at pH 5.0 but not much further once the concentration was in excess of 1% (0.17M/dm^3). Barbut (1995) and Ikeda & Foegeding (1999)

severally reported a decrease in the water holding capacity with the increase in salt concentration as a result of the change in the gel network from a compact fine stranded gel to a coarse, particulate gel with a large effective pore size and a lower resulting water holding capacity. Barbut further attributed the particulate gel to the formation of large aggregates as a result of the production of thicker and larger protein strands. It was also shown that the optimum level for the maximum effect of NaCl on gel strength and water holding capacity is 0.2M. Ikeda & Foegeding (1999) also showed that water holding capacity dwindles with an increase in salt concentration. Chantrapornchai & McClements (2002) attributed the decrease in water holding capacity to the change in the structure of the gel from a fine-stranded gel to a particulate gel. The effect of the change was to increase the pore size and thereby capillary pressure.

Fracture Properties

Neither of the fracture properties showed massive changes as the concentration of the NaCl was altered (Figs 4.17-E & 4.17-F). The binders with the highest ratio of albumen showed the biggest stress fracture.

Figure 4.17-E

The Effect of NaCl on Fracture Strain

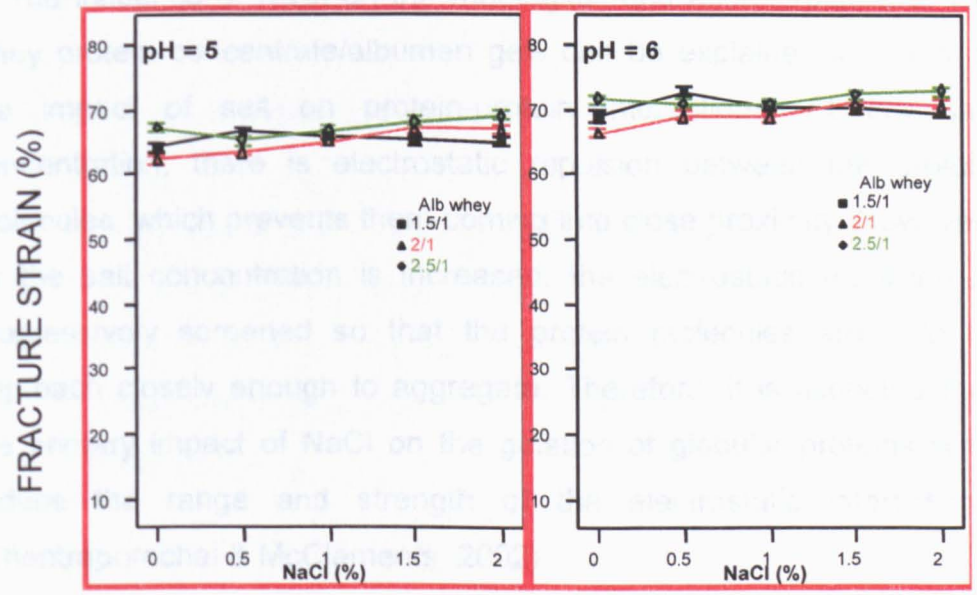
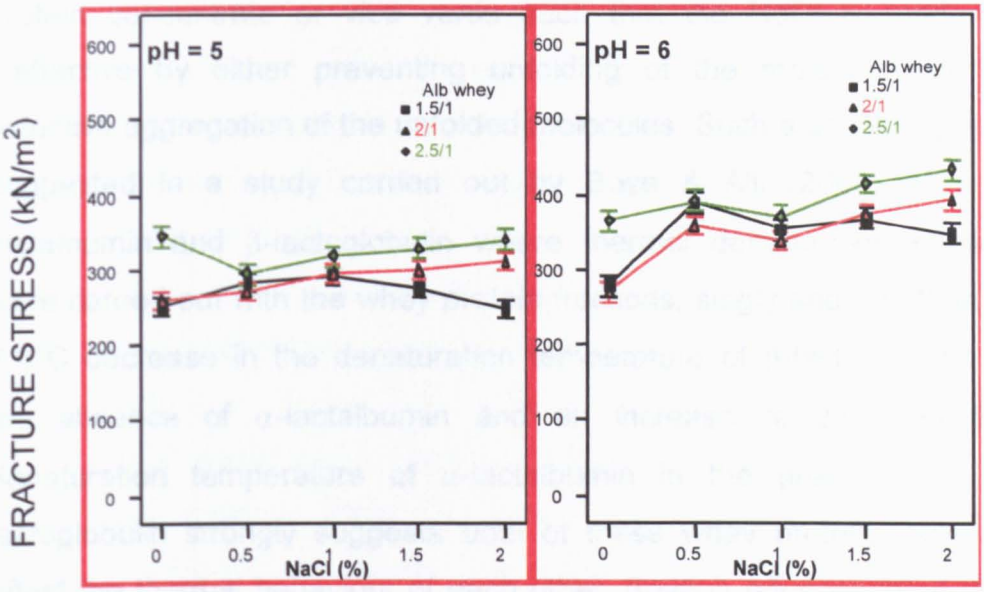


Figure 4.17-F

The Effect of NaCl on Fracture Stress



Ikeda & Foegeding (1999) noted that at concentrations of monovalent cations higher than 0.1M/dm^3, the network is a mixture of fine strands and spherical aggregates that are high in stress and minimum in strain at the point of fracture. In an earlier study, Kuhn & Foegeding (1991) had shown that in a sample of whey protein gel, NaCl had the consequence of a sharp increase in the fracture stress up to a concentration of about 0.05M – 0.075M. Above this concentration, there was a decrease in the fracture stress. By contrast, the fracture strain decreased rapidly with an increase in the NaCl up to about 0.1M.

The influence of NaCl on the rheological and microstructure of the whey protein concentrate/albumen gels can be explained in terms of the impact of salt on protein-protein interactions. At low salt concentration, there is electrostatic repulsion between the protein molecules, which prevents them coming into close proximity. However, as the salt concentration is increased, the electrostatic repulsion is progressively screened so that the protein molecules are able to approach closely enough to aggregate. Therefore, it is accepted that the primary impact of NaCl on the gelation of globular proteins is to reduce the range and strength of the electrostatic interactions (Chantrapornchai & McClements, 2002).

In the present study, it has not been ascertained whether there is a synergistic protection of the egg albumen protein molecules by whey protein concentrate or vice versa such that the NaCl is rendered ineffective by either preventing unfolding of the molecules or by selective aggregation of the unfolded molecules. Such a possibility was suggested in a study carried out by Boye & Alli (2000) with α-lactalbumin and β-lactoglobulin where thermal denaturation studies were carried out with the whey protein fractions, singly and together. A 2.8°C decrease in the denaturation temperature of β-lactoglobulin in the absence of α-lactalbumin and an increase of 2.5°C in the denaturation temperature of α-lactalbumin in the presence of β-lactoglobulin strongly suggests both of these whey protein fractions affect the thermal behaviour of each other. Boye & Alli postulated that the unfolding of the α-lactalbumin on denaturation might initiate cross-linking interactions with the exposed sites on the β-lactoglobulin molecule thereby speeding up the unfolding of the β-lactoglobulin. Even in the presence of other ingredients such as sodium bicarbonate and sodium ascorbate, the presence of α-lactalbumin still decreased the thermal stability of the β-lactoglobulin. Howell & Laurie (1984) proposed that the synergistic interactions between compatible globular proteins depended on the degree of unfolding of the ***individual proteins*** (**italics mine**) in the mixture which governed optimum exposure of specific groups and hence the optimum interaction.

4.18 Conclusions/Key Findings

I. There were **significant interactions** between virtually all the main effects of one or more of the response variables.

II. In the presence of other materials, **the 2/1 ratio of albumen** to whey protein concentrate as discussed in Chapter 3 **did not always have the highest or lowest value** of a particular gel characteristic.

III. The **pH generally had the biggest effect** on the gel characteristics probably because most of the experiments were carried out in the isoelectric region.

IV. The effect of sugars on gel properties was generally smaller than the effect of pH. **Lactose however, did produce a dramatic reduction in fracture strain** at concentration in excess of 0.5%.

V. Of all the materials assessed, the starches exhibited the largest effect on the gel hardness.

VI. The two major hydrocolloids studied i.e. **pectin and methylcellulose, showed different effects on the gel properties**. Although both have affinity for water, methylcellulose appeared to be the stronger one as the gels in which it was present **changed from exuding water to imbibing water** at concentrations in excess of 0.5%. The effects on residual stress and fracture strain were also markedly different for both materials.

VII. The relationship between **lecithin concentration and the gel characteristics appeared to be non linear**. The peak/trough occurred at about 1% lecithin concentration.

VIII. **The milk-derived ingredients of lactose, GMP and casein all had differing effects on the gel properties**. Increased levels of casein consistently increased the elastic properties but reduced the plastic and fracture properties. Increased GMP had a mixed effect on the elastic properties but very little effect on the viscous properties or fracture properties. Addition of varying levels of lactose had little effect on the elastic and plastic properties but a marked effect on the fracture properties.

CHAPTER 5

CHARACTERIZATION OF MOLECULAR INTERACTIONS WITH PROTEIN PERTURBING AGENTS

Summary

The results of the gel dissolution by protein perturbing agents are described and discussed in this section in the context of the experiments performed as described in Section 2. Buffer solutions for the protein dissolution tests were prepared as described in Section 2.4. The gel samples were prepared and dissolved in the buffer solutions as described in Section 2.12.

The gel samples selected for dissolution tests were high methoxyl pectin, high amylose starch (Hylon VII) and NaCl.

Results and Discussion

Figure 5-A

Gel Protein Dissolution with Different Buffer Solutions (pH 5)

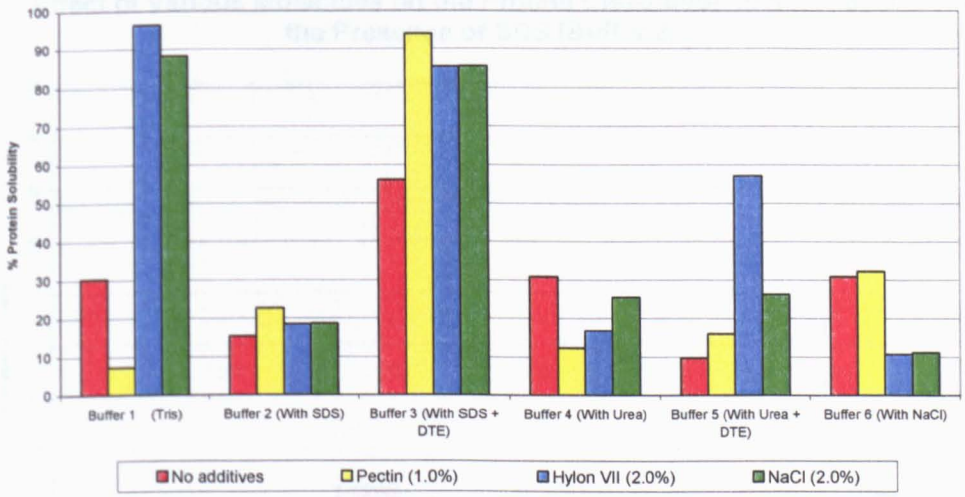
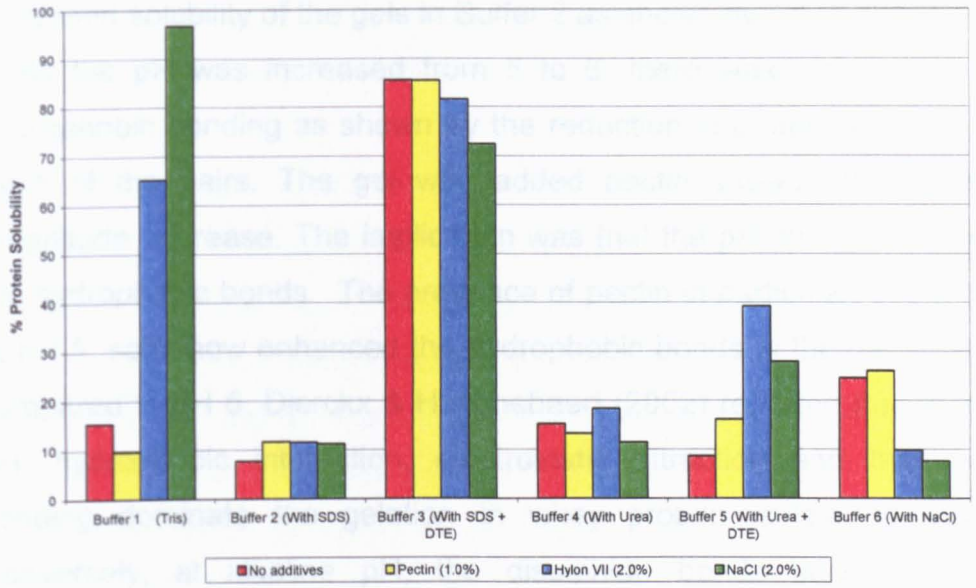


Figure 5-B

Gel Protein Dissolution with Different Buffer Solutions (pH 6)

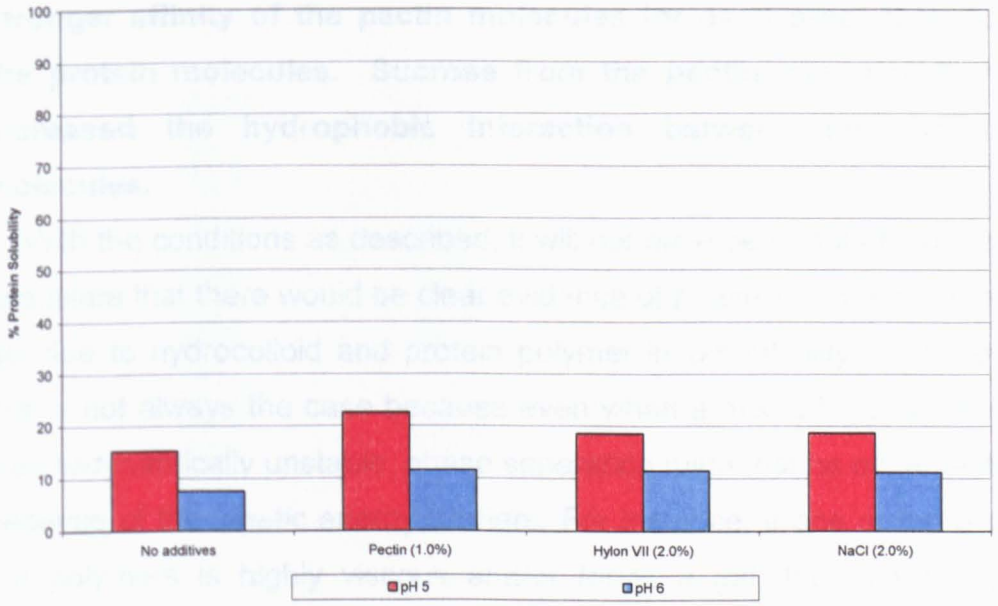


5.1 Hydrophobic Bonds

When SDS was added to the buffer solutions, (Buffer 1 to Buffer 2) there was a reduction in protein solubility for the 'no additive' gel as well as the starch and salt gels at both pH 5 and pH 6 (Fig. 5-A and B). The pectin infused gel was the exception. There was a slight increase

Figure 5.1-A

Effect of Various Molecules on the Protein Dissolution in a Gel System in the Presence of SDS (Buffer 2)



in the protein solubility especially at pH 5. It may be that the lower pH favoured pectin-pectin interactions. This was borne out by the increased solubility of the gels in Buffer 2 as shown by Fig. 5.1-A.

As the pH was increased from 5 to 6, there was a reduction in hydrophobic bonding as shown by the reduction in protein solubility in each of the pairs. The gel with added pectin showed the largest magnitude decrease. The implication was that the pH change affected the hydrophobic bonds. The presence of pectin in particular, in the gel at pH 5, somehow enhanced the hydrophobic bonds in the gel system compared to pH 6. Dierckx & Huyghebaert (2002) reported that at low pH, hydrophobic interaction, electrostatic attraction and hydrogen bonding dominate the gelation in whey protein isolate solutions. Conversely, at alkaline pH, the disulphide bonds become more dominant. Back *et al.,* (1979) had previously shown that sucrose could enhance the hydrophobic interactions between protein molecules. Other studies have also shown that junction zones in gels of high methoxyl pectin are stabilized by hydrophobic interactions between the methyl ester groups (Walkinshaw & Arnott, 1981; Oakenfull & Scott, 1984). **In the light of these observations and the fact that commercial pectin samples are standardized with sucrose, it is likely that the increase in hydrophobic interactions detected with the pectin-infused gel in the present study at pH 5 was due to the stronger affinity of the pectin molecules for each other than for the protein molecules. Sucrose from the pectin may also have increased the hydrophobic interaction between the protein molecules.**

With the conditions as described, it will not have been out of place to speculate that there would be clear evidence of phase separation in the gel due to hydrocolloid and protein polymer incompatibility. However, this is not always the case because even when a mixed biopolymer is thermodynamically unstable, phase separation might not be observable because of the kinetic energy barriers. For instance, if one or more of the polymers is highly viscous and/or forms a gel, the rate and/or

extent of the phase separation may be slow (Bryant & McClements, 2000a).

At pH 6, there was no noticeable effect from any of the three additives on the protein solubility.

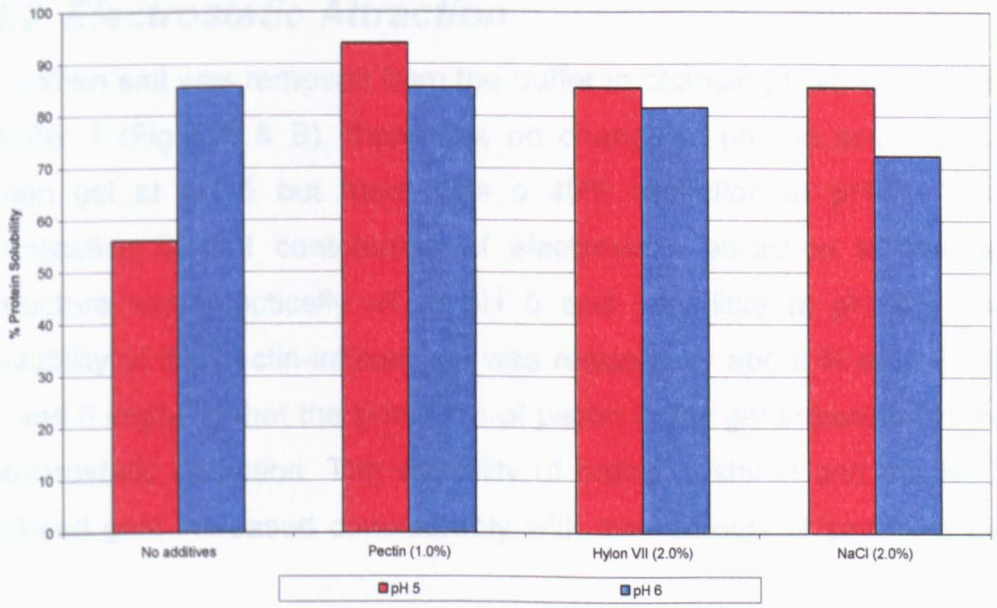
5.2 Disulphide Bonds

When the DTE was removed, as in a change from Buffer 3 to Buffer 2 Fig. 5-A & B), there was a significant reduction in protein solubility for all four gels. The change occurred at both pH 5 and pH 6 **showing that disulphide bonds were mainly responsible for maintaining the gel structure.** All three infusions i.e. high methoxyl pectin, NaCl and Hylon VII appeared to lead to an enhancement of the disulphide bonds at pH 5 as shown by the higher solubility of the gel as compared to the 'no additive' gel (Fig. 5.2-A)

In a similar buffer, when DTE was removed, as in a change from Buffer 5 to Buffer 4 (Fig. 5-A & B), there was reduction in the solubility of only the Hylon starch-infused gel at pH 5. The other infused gels as well as the 'no additive' gel displayed no effect. At pH 6, the Hylon gel and the NaCl gel both exhibited a reduction in protein solubility.

Figure 5.2-A

Effect of Various Molecules on the Protein Dissolution in a Gel System in the Presence of SDS and DTE (Buffer 3)



Starches do not normally contain sulphydryl or disulphide groups and are not normally expected to participate in the formation of inter- or intra-molecular disulphide bonds. However, it cannot be ruled out that attractive interactions occur between polymers due to patches of charged residues on the protein surface. **On the other hand, could this be an example of what is described by Aguilera & Baffico (1997) as an 'active phase-separated gel'?**

Fig 5.2-A also demonstrated that as the pH was increased in the from a value of 5 to 6, the disulphide bonds were reduced very slightly as shown by a small reduction in protein solubility of the three infused gels in Buffer 3. Buffer 3 will attack both hydrophobic and disulphide bonds. The plain gel is the exception: the increased protein dissolution showed that the disulphide bonds increased. Neutral and alkaline pH values tend to promote disulphide bond formation. Although the magnitude of the difference in soluble protein between the pH values was small, it is possible that there was an interaction between the added macromolecules at pH 6. The effect of this interaction may have been to interfere with the ability of the protein molecules to fully unfold and hence reduce the extent of the thiol – disulphide reaction. When there were no additives, as in the plain gel, this was not the case.

5.3 Electrostatic Attraction

When salt was removed from the buffer in changing from Buffer 6 to Buffer 1 (Fig 5-A & B), there was no change in protein solubility for plain gel at pH 5 but there was a 40% reduction at pH 6. The implication is that contribution of electrostatic attraction to the gel structure was practically nil at pH 5 and very little at pH 6. The solubility of the pectin-infused gel was reduced by about ⅔ at both pH 5 and 6 implying that the presence of pectin in the gel impacted on the electrostatic attraction. The solubility of both the starch and the NaCl infused gels increased considerably with the removal of salt from the

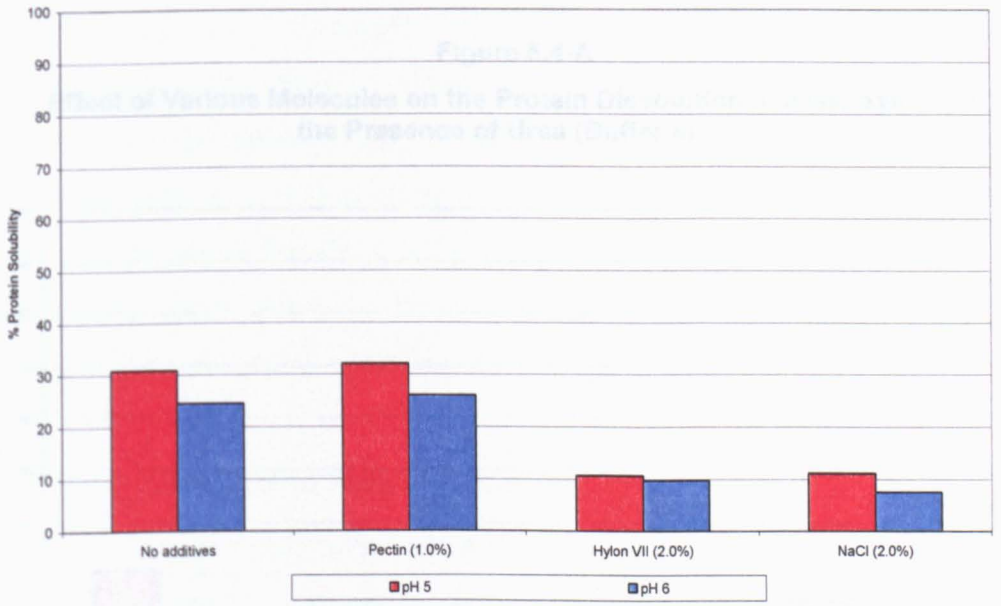
buffer. No explanation can be provided for this observation at the present time.

When the plain gel was compared with the infused gels at pH 5, (Fig 5.3-A), it was observed that both Hylon VII and NaCl reduced the solubility by about a third, showing that there was a slight reduction in the electrostatic attraction between the protein molecules as a result of the added molecules. This meant that when either the starch or NaCl was introduced to the gel, a measure of electrostatic attraction came into force. Pectin did not seem to change the protein solubility.

As the pH was increased from 5 to 6, the plain gel suffered a slight reduction in electrostatic attraction as evinced by the reduction in protein solubility. The reduction in electrostatic attraction was exacerbated by the starch and the NaCl in a pattern similar to what was seen at pH 5. The effect of salt addition as a perturbing agent can generally be accounted for by screening charges, which leads to a decreased electrostatic attraction between the polymer chains (Schmelter *et al.*, 2001).

Figure 5.3-A

Effect of Various Molecules on the Protein Dissolution in a Gel System in the Presence of NaCl (Buffer 6)



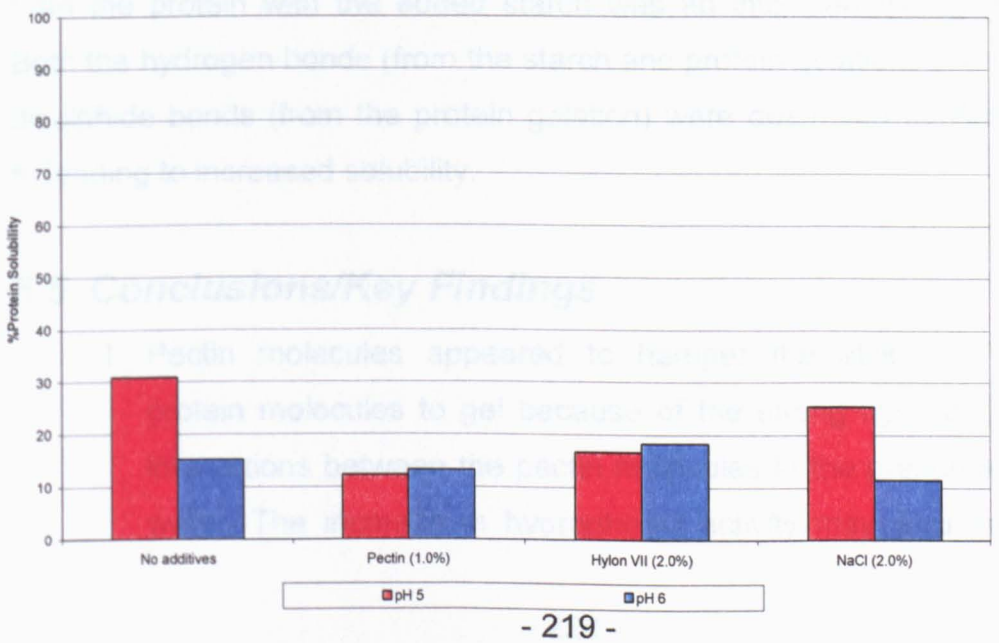
5.4 Hydrogen Bonding

When urea was removed from the buffers as shown by changing from Buffer 4 to Buffer 1 (Fig 5-A & B), there was only a small change in protein solubility for the plain gel at either pH. The implication is that hydrogen bonding did not contribute significantly to the network of the plain gel. The solubility of the pectin-infused gel was reduced very slightly at both pH 5 and 6 implying that the presence of pectin in the gel impacted somewhat on the hydrogen bonding. With Hylon VII and NaCl however, the elimination of urea from the buffer led to a massive increase in protein solubility as compared to the plain gel. No explanation can be provided at present for this observation.

However, when the gels with added material were compared to the 'no additive' gels at pH 5 as highlighted in Fig. 5.4-A, it was observed that the Hylon VII, and especially the pectin, reduced the protein solubility implying a reduction in the hydrogen bonding. Therefore with the presence of these two materials in the gel, part of the overall structure was now maintained by hydrogen bonding. So while hydrogen bonding was not generally a major contributor to network of the plain gel structure, pectin reduced the little there was by about two-thirds and the Hylon VII starch reduced it by about half.

Figure 5.4-A

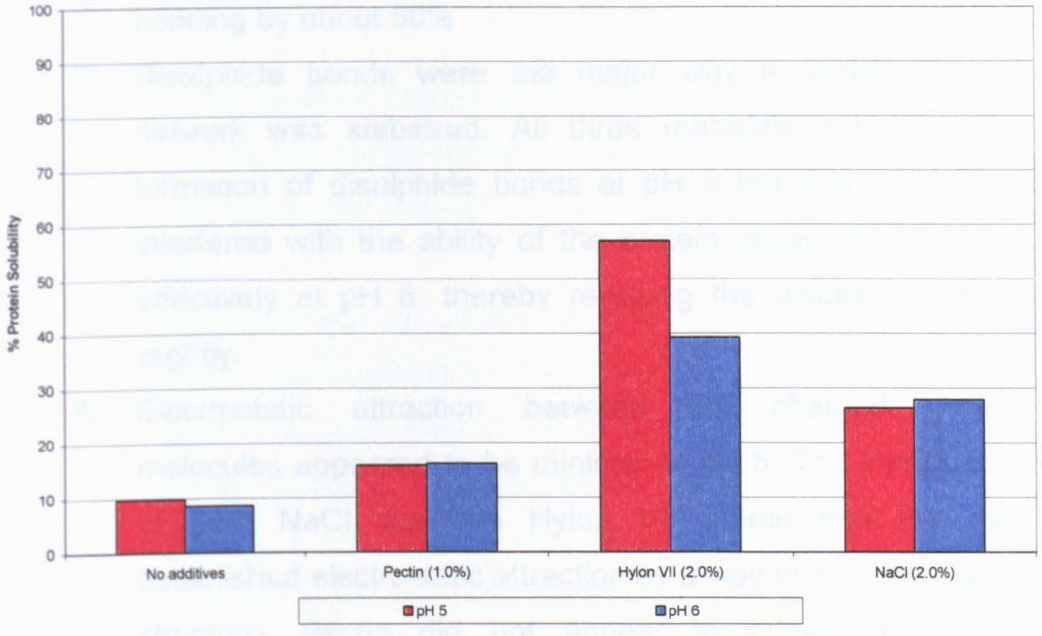
Effect of Various Molecules on the Protein Dissolution in a Gel System in the Presence of Urea (Buffer 4)



Increasing the pH from 5 to 6 had the effect of reducing the hydrogen bonding in the plain gel. At this pH, there was very little effect from any of the additives on hydrogen bonding.

Figure 5.4-B

Effect of Various Molecules on the Protein Dissolution in a Gel System in the Presence of Urea + DTE (Buffer 5)



When DTE was added to the urea buffer i.e. a change from Buffer 4 to 5, there was increased protein solubility for all three infused gels at both pH values. The Hylon VII infused gel exhibited the biggest magnitude change. The DTE and urea buffer will attack both hydrogen bonds and disulphide bonds. The hypothesis is that the gel formed from the protein with the added starch was an inter-penetrating gel. Both the hydrogen bonds (from the starch and protein gelation) and the disulphide bonds (from the protein gelation) were destroyed by Buffer 5, leading to increased solubility.

5.5 Conclusions/Key Findings

I. Pectin molecules appeared to hamper the ability of the protein molecules to gel because of the strong hydrophobic interactions between the pectin molecules in the presence of water. The increase in hydrophobic activity may also have

been due to sucrose from the pectin affecting the protein-protein interaction.

II. Hydrogen bonding only played a small role in the protein gel structure. Nevertheless, at pH 5, the incorporation of pectin into the gel system reduced hydrogen bonding by about 60% whilst the Hylon VII high amylose starch reduced hydrogen bonding by about 50%

III. Disulphide bonds were the major way in which the gel network was stabilized. All three materials enhanced the formation of disulphide bonds at pH 5 but they may have interfered with the ability of the protein molecules to unfold effectively at pH 6, thereby reducing the disulphide bonds slightly.

IV. Electrostatic attraction between the charged protein molecules appeared to be minimal at pH 5. The introduction of both NaCl and the Hylon VII starch into the gels established electrostatic attraction as a way of maintaining gel structure. Pectin did not appear to affect electrostatic attraction in the gel.

CHAPTER 6

CONFOCAL LASER SCANNING MICROGRAPHS OF SELECTED GEL SAMPLES

Summary

Not all of the samples manufactured from different materials could be analysed using the confocal laser scanning microscope due to time constraints. The samples selected for examination of the gel structure were as follows:

I. **Binder 2/1 with no added material at pH 5 and 6** – The 2/1 sample was selected for two reasons (1) it represented the extreme point in most of the original TPA tests described in Chapter 3 and (2) it served as the 'control'.

II. **Binder 2/1 with rice starch (2%) at pH 5 and 6 and binder 2/1 with high amylose starch** – These samples were selected because they produced increasing hardness with the protein gel. It was also important to be able to perceive if there were any in the effect of the two starch products on gel characteristics.

III. **Binder 2/1 with high methoxyl pectin (0.5% and 1%) at pH 5 and 6** – Due to the significant change in most of the gel attributes, the pectin infused gel was selected for CLSM analysis. The two different levels of pectin addition were assessed to determine whether the changes observed with gel deformation test could be explained at microscopic level.

IV. **Binder 2/1 with $CaCl_2$ (2%) at pH 5 and 6** – Of the two gel samples with added salts, the $CaCl_2$ infused gel was selected because it exhibited a bigger and more unusual effect than NaCl on some of the gel properties, notably expressible water.

V. **Binder 2/1 with lactose (2%) at pH 5 and 6** – The effects of both lactose and dextrose were similar but the lactose had a much bigger effect on the fracture strain of the gel and so was selected for CLSM for this purpose.

The gels from the binder samples were prepared as described in Section 2.13. All the micrographs shown in this chapter were derived from the pre-gelled samples made as described in Section 2.13.1. The results from the binders gelled in situ were not shown due to difficulties associated with the presentation of the gel samples.

Results and Discussion

6.1 2/1 Protein Gel With No Added Material

Figure 6.1-A & B show that at a microscopic level, big differences exist in the microstructure. In the pH 6 sample, the fluorescence was evenly distributed in the gel structure. It looked smoother and was homogenous with very few, if any of the dark patches that characterized the gel at pH 5. This was indicative of fewer particles or aggregates and the gel is likely to be a fine-stranded gel. At pH 5, the microstructure changed dramatically. There were a lot of dark patches in the gel made at pH 5. The void spaces, as typified by the dark patches i.e. non-protein material, were bigger. These were almost certainly water molecules that had not been evenly incorporated into the gel network. The brightly coloured patches were almost certainly composed of aggregates of the protein as part of a phase- separated gel but more likely a particulate gel.

Figure 6.1-A

CLSM Image of Albumen/Whey Protein Gel With No Added Material (pH 5)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)

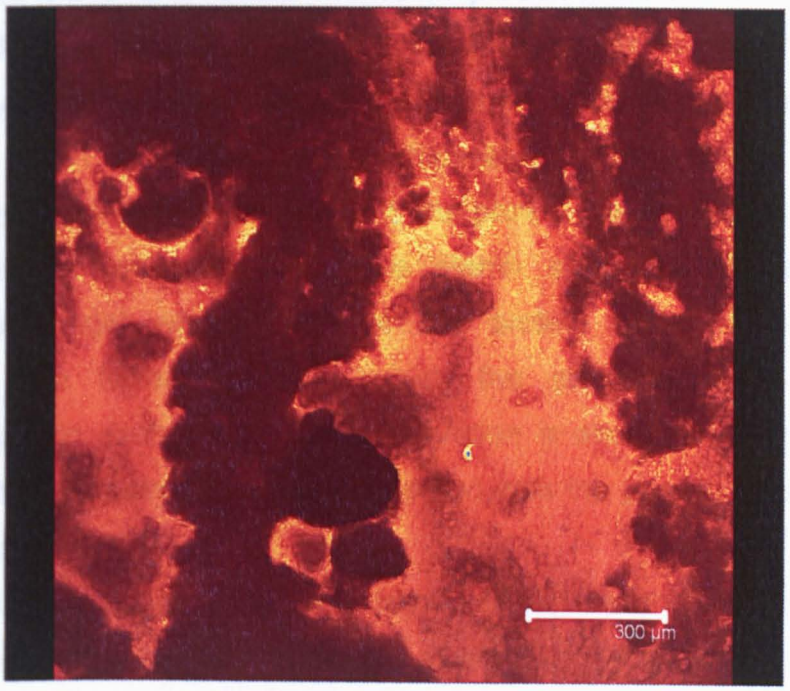
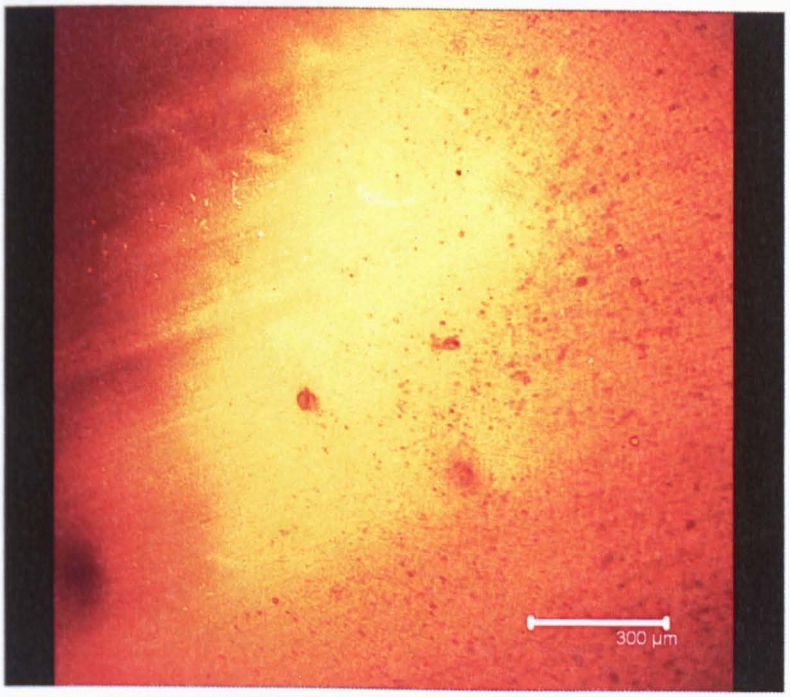


Figure 6.1-B

CLSM Image of Albumen/Whey Protein Gel With No Added Material (pH 6)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)



6.2 2/1 Protein Gel With Rice Starch (2%)

In Figures 6.2-C and D the individual particles were a lot more visible at 300 microns. The two gel samples looked homogenous. Interestingly, the aggregates at pH 6 appeared to be larger than the aggregates at pH 5. The non-protein material, as shown by the dark patches, was more evenly dispersed in the protein gel at pH 5 than at pH 6. The gel presented with rice starch appeared to be an interpenetrating gel where each of the protein and rice starch molecules both formed gels in which the strands/particles are interspersed with each other. This observation is almost certainly the reason for the increased hardness observed with the protein gels with added starch.

Although there appeared to be a degree of separation between the protein and starch molecules in the gel at pH 6 as compared to pH 5, the fact that there were no major differences observed in the TPA hardness (Fig 4.6-A) may imply that the differences were only superficial. However, there were differences in TPA springiness (Fig. 4.6-B) and residual stress (Fig.4.6-G) at pH 5 and 6, which may be explained by the separation between the starch and protein molecules.

Figure 6.2-A

CLSM Image of Albumen/Whey Protein Gel With 2% Rice Starch (pH 5)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)

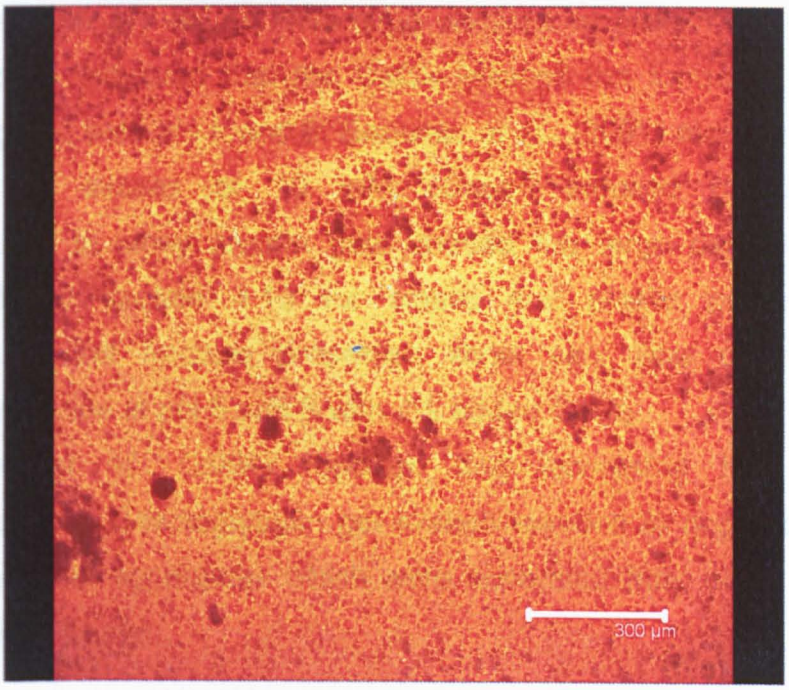
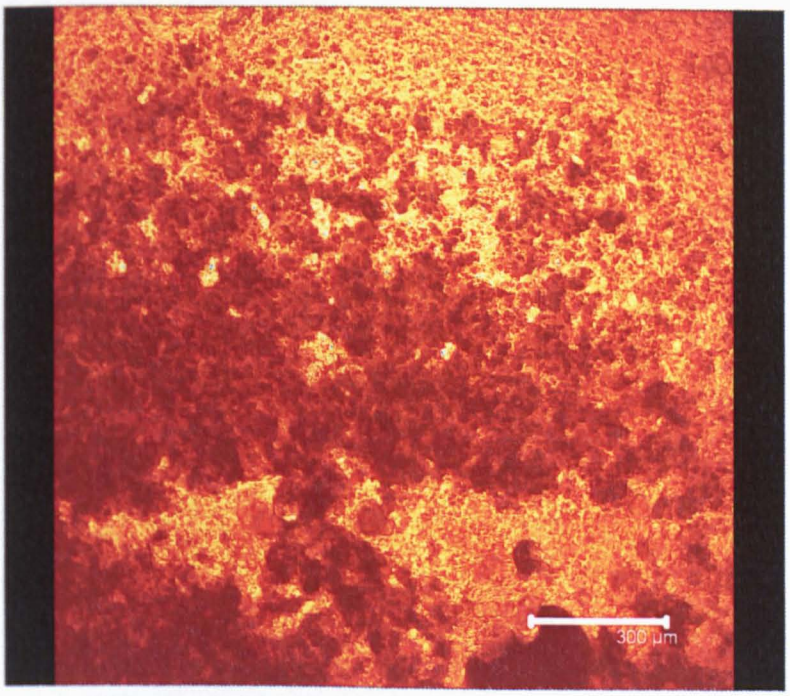


Figure 6.2-B

CLSM Image of Albumen/Whey Protein Gel With 2% Rice Starch (pH 6)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)



6.3 2/1 Protein Gel With $CaCl_2$ (2%)

There did not appear to be much difference between the gel structures at both pH values. The large sections of protein and non-protein patches indicated that the $CaCl_2$ induced the formation of a particulate gel at both pH 5 and 6. Beaulieu *et al.,* (2001) reported that dispersions in excess of 10 mM calcium induced particulate microstructures in whey protein gels. Indeed the large deformation tests results in section 4.10 for elastic and plastic properties, microstructure and fracture properties show very little difference between gels made at pH 5 and 6 with 2% added $CaCl_2$.

Sok Line *et al.,* (2004) reported that increasing the Ca^{2+} concentration in a β-lactoglobulin gel system resulted in larger pores and larger protein aggregates which were separated from each other by the aqueous phase.

Figure 6.3-A

CLSM Image of Albumen/Whey Protein Gel With 2% $CaCl_2$ (pH 5)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)

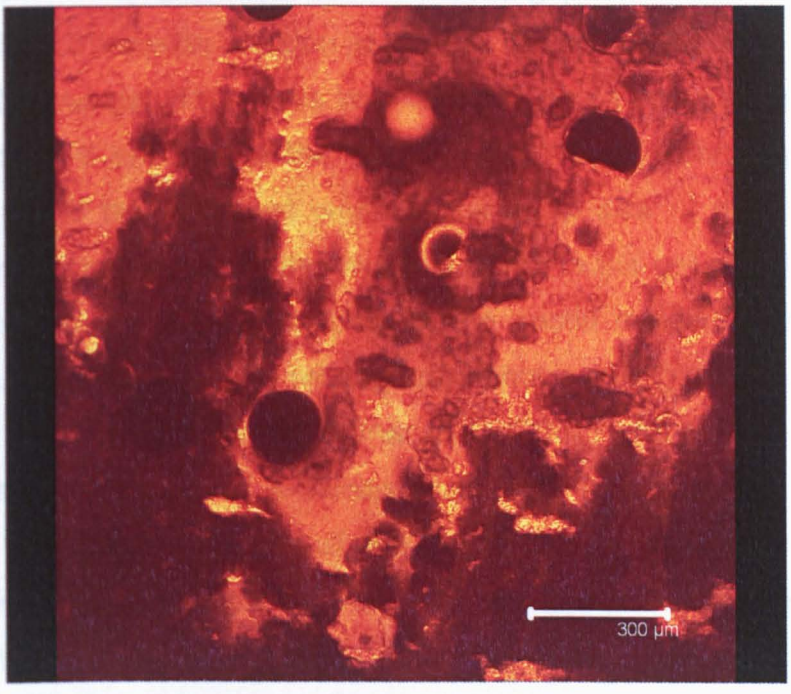
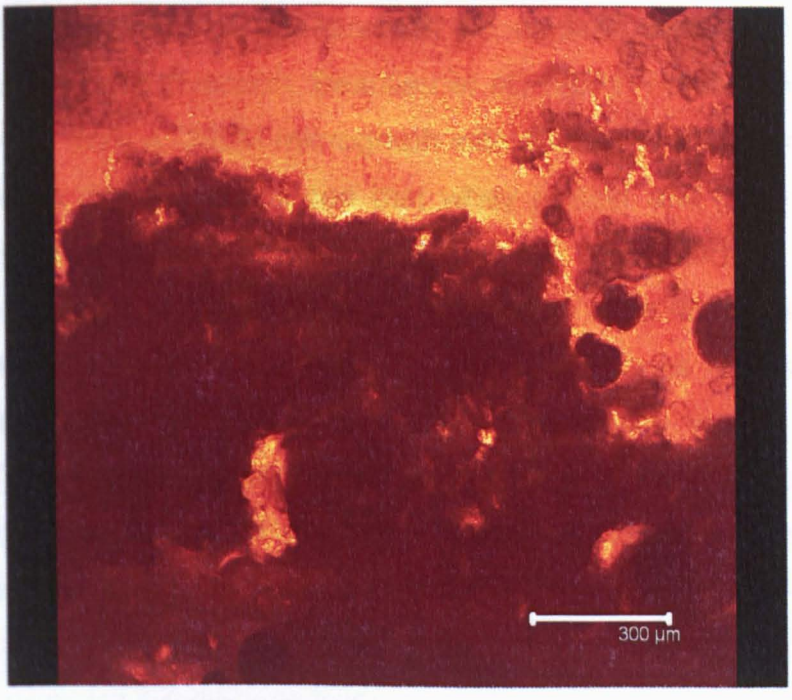


Figure 6.3-B

CLSM Image of Albumen/Whey Protein Gel With 2% $CaCl_2$ (pH 6)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)



6.4 2/1 Protein Gel With High Methoxyl Pectin (0.5 % & 1%)

Figures 6.4-A - D show the effect of adding pectin to the binder system. With 0.5% pectin addition at pH 5.0, the gel appeared to be a protein continuous gel. There was very little difference between the gel at pH 5 and pH 6 except that the non-protein particles were larger at the higher pH. It is not known whether the large size of the pectin gelled particles contributed to the tortuous paths in the gel at pH 5 or whether this was just an overlapping of the pectin molecules in the micrograph. Nevertheless, the micrographs shown in Fig. G - J were very similar to those obtained by Hemar *et al.* (2002) who carried out a series of experiments with κ-carrageenan and milk protein. They concluded that the separation of the gel phases was induced by depletion flocculation caused by the κ-carrageenan molecules in the aqueous phase.

At the higher concentration of pectin (1%), the gel structure appeared to be dominated by the pectin molecules/aggregates, with only small a small proportion of the gel being the protein. In the gel at pH 6 in particular, it appeared that the protein molecules have coated the pectin molecules/aggregates. It can be inferred that the gel system was now a pectin-continuous gel with the protein strands/aggregates acting as filler particles. It appeared to be a clear case of phase separation and phase inversion. Beaulieu *et al.*, (2001) made a similar judgment when from a CSLM micrograph with a whey protein and low methoxyl pectin gel and concluded that whey protein and pectin are thermodynamically incompatible and lead to phase separation.

Although samples at higher pectin concentration were not examined by CLSM, the results from the large deformation tests as reported in Section 4.4b suggested that further phase separation would have occurred. The increase in fracture strain (Fig. 4.4-E) when the pectin concentration is in excess of 1% at pH 6 may be due to incomplete phase separation due to increased phase viscosity and gelation rate.

DeMars & Ziegler (2001) reported such an event in a study of the phase separated gelation of pectin and gelatin.

Figure 6.4-A

CLSM Image of Albumen/Whey Protein Gel With 0.5% High Methoxyl Pectin (pH 5)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)

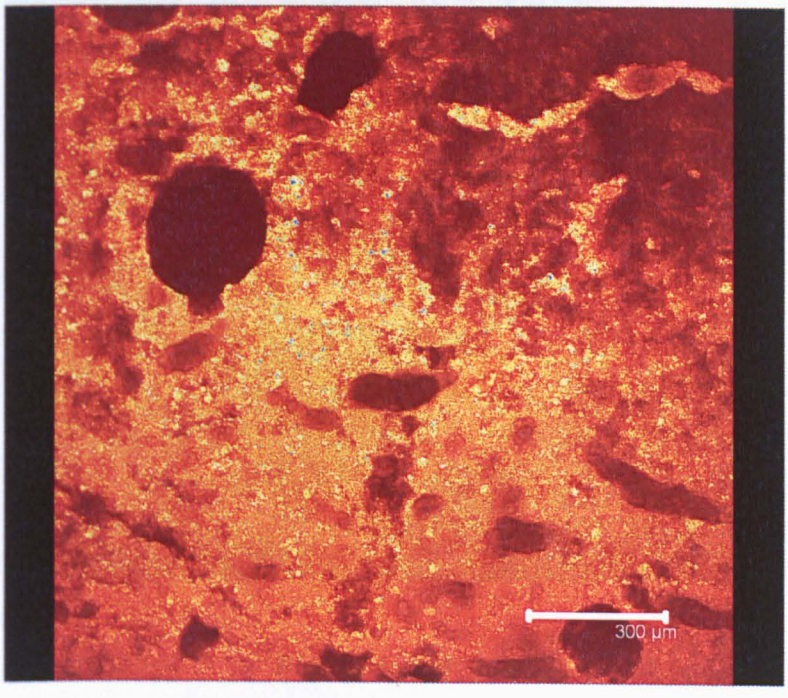


Figure 6.4-B

CLSM Image of Albumen/Whey Protein Gel With 0.5% High Methoxyl Pectin (pH 6)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)

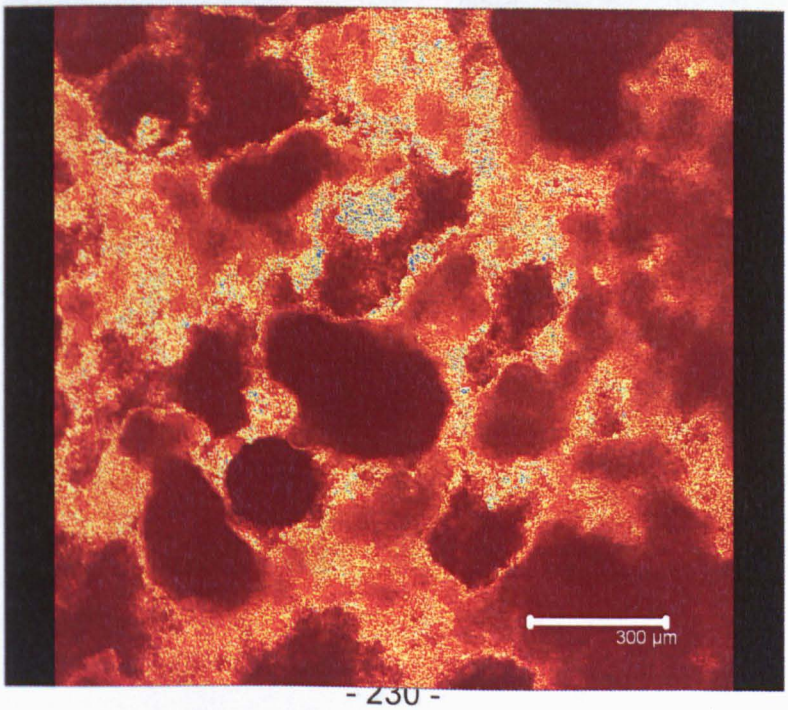


Figure 6.4-C

CLSM Image of Albumen/Whey Protein Gel With 1.0% High Methoxyl Pectin (pH 5)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)

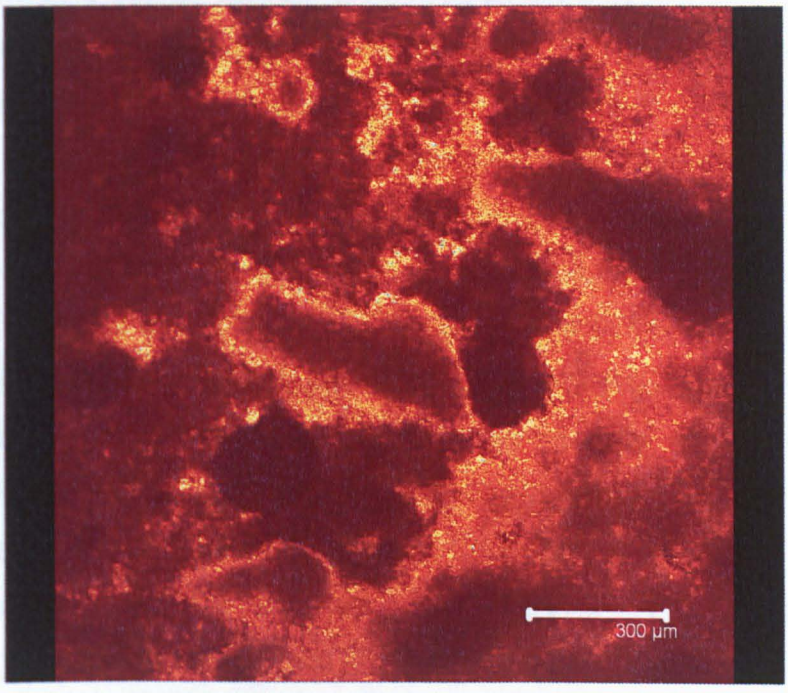
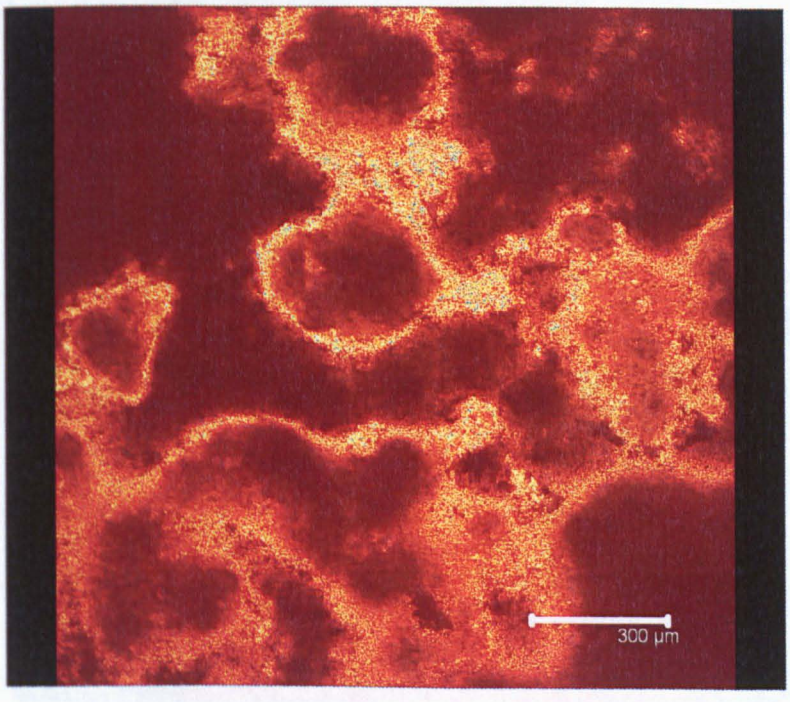


Figure 6.4-D

CLSM Image of Albumen/Whey Protein Gel With 1.0% High Methoxyl Pectin (pH 6)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)



6.5 2.1 Protein Gel With High Amylose Starch (2%)

Figures 6.5-A and B show the effect of the high amylose starch in the binder gel system. At pH 5, the starch (non-protein) granules were clearly visible and evenly spread out through the gel structure. Due to the fact that there was very little difference in the TPA hardness for the high amylose infused starch at 2% at either pH (Fig. 4.7–A) it could have been theorized that the starch particles were behaving as an inactive filler within the protein gel matrix. However, all the other tests carried out and reported in Section 4.7, showed differences between the two gel samples at pH 5 and 6 (2% dosage) with the exception of the TPA hardness. TPA Springiness, fracture strain and fracture stress were higher in the gel at pH 6, whereas the residual stress, water uptake and expressible water were higher in the sample at pH 5. The combined evidence suggests that at pH 5, the starch particles were behaving as filler particles, but that at pH 6 there was evidence of some other *modus operandi*, most likely phase separation between the starch gel and the protein gel.

Figure 6.5-A

CLSM Image of Albumen/Whey Protein Gel With 2.0% High Amylose Starch (pH 5)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)

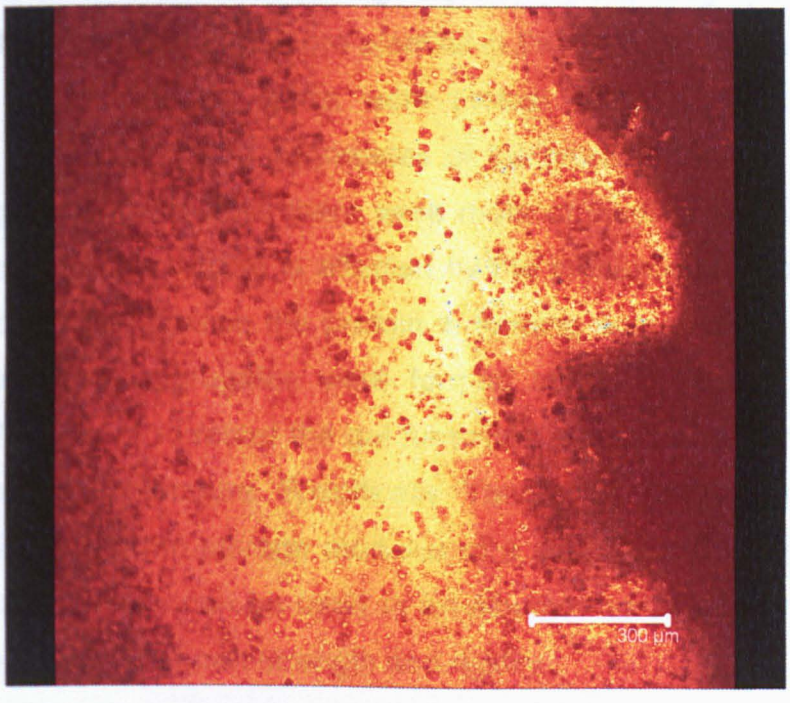
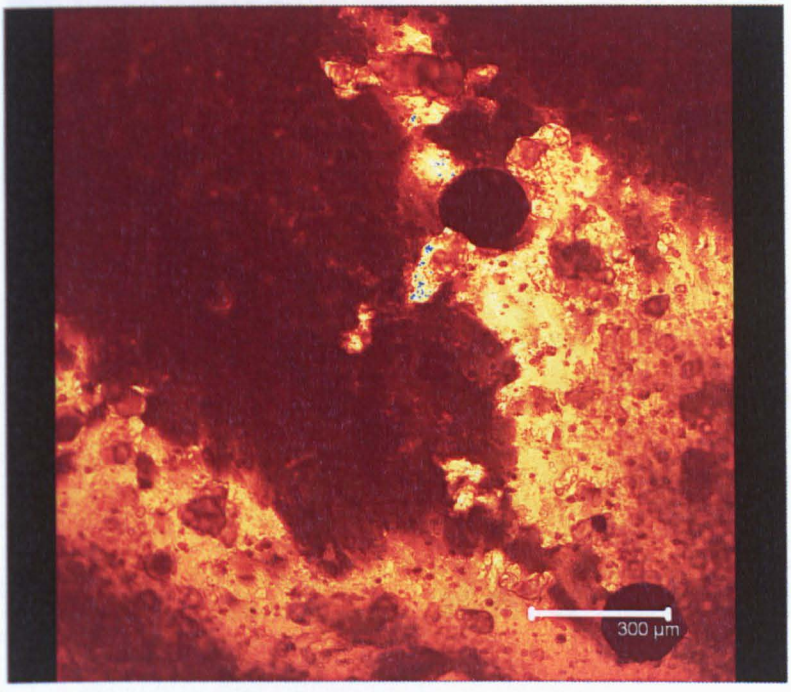


Figure 6.5-B

CLSM Image of Albumen/Whey Protein Gel With 2.0% High Amylose Starch (pH 6)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)



6.6 Protein Gel With Lactose (2%)

There was a mixture of dark and light specs in the micrograph (Fig. 6.6-A and B). The specs were more plentiful in the pH 6 gel than in the pH 5 gel. The fluorescent specs were protein particles and more specifically, were likely to be aggregates of the protein formed in *situ* during the gelling process. As there were more of them at the higher pH, it is likely that the lactose is affected by the presence of acid and was not allowed to influence the protein in forming aggregates at this pH. Perhaps more aggregates were formed at pH 6. The normal tendency of sugars is to retard the gel formation process and increase the denaturation temperature. Specifically, Yang *et al.,* (2004) showed that lactose affected the storage modulus of a starch-sucrose system and stated that lactose can be used in an albumen and whey protein concentrate system to impart strength and rigidity.

Figure 6.6-A

CLSM Image of Albumen/Whey Protein Gel With 2% Lactose (pH 5)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)

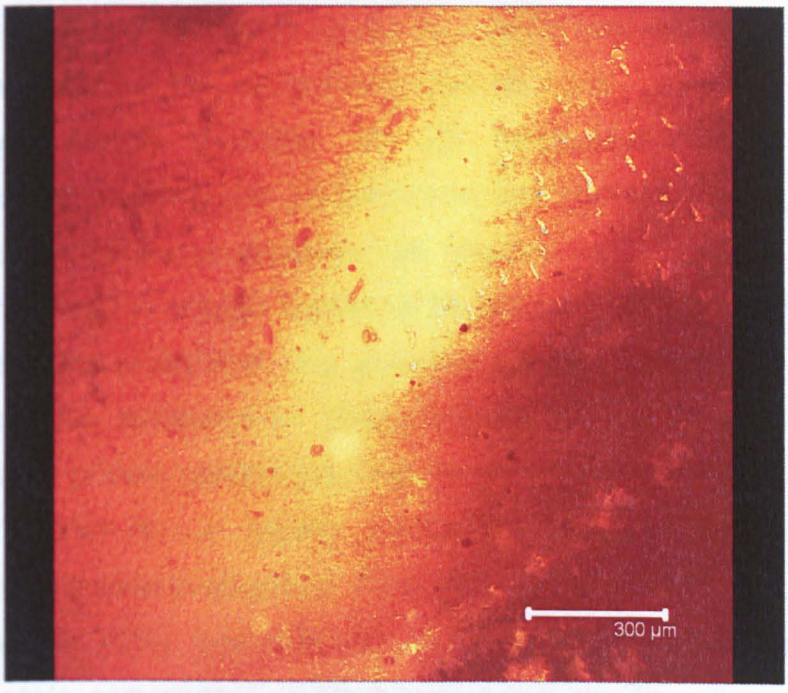
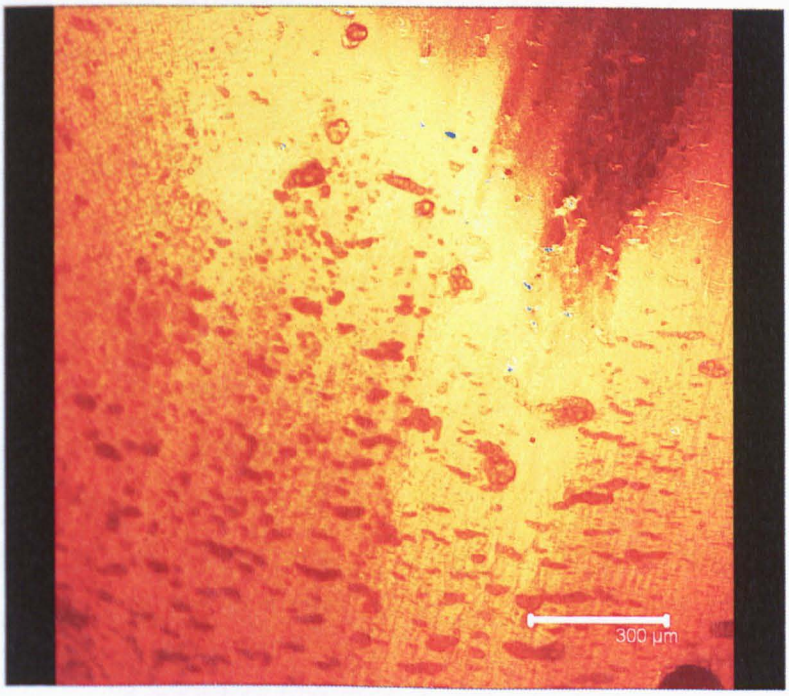


Figure 6.6-B

CLSM Image of Albumen/Whey Protein Gel With 2% Lactose (pH 6)

(Stained with 0.0005% Rhodamine B. Protein phase is orange)



6.7 Conclusion/Key Findings

I. There was an interaction between pH and the added materials on the microstructure of the gel systems.

II. At pH 5, the gels were mostly of a particulate microstructure while at pH 6, they were mostly fine-stranded.

III. **Rice starch addition at 2% produced mostly an interpenetrating gel network** whereas high amylose starch produced a phase separated gel network.

IV. Incorporation of **high methoxyl pectin** into the binder system led to the production of a **phase-separated network**. There was also **evidence of phase inversion:** the gel changed from a protein continuous network (at a pectin concentration of 0.5%) to a pectin continuous network (at pectin concentration in excess of 1%).

V. Lactose (2%) appeared to influence the **production of protein aggregates** within the gel structure. The aggregates were particularly evident at pH 6.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS FOR FURTHER WORK

7.1 Albumen and Whey Protein Concentrate

The present study has shown that there exist statistically significant interactions between whey protein concentrate and albumen, when used in combination in a heat-induced gel. To date, there are only a handful of studies that exist on the interaction of whey protein concentrate and albumen in a mixed gel system. Fewer still exist on the interaction between a whey protein concentrate/albumen blend and other materials. In view of the importance of gel systems in the production of food, both domestically and industrially, this is a surprise. I have been able to show that there is an optimum blend of whey protein concentrate to albumen that exists in the region of 2:1.

As expected, pH plays a key role in determining the characteristics of the blended gel system. At the optimum pH, it was possible to make a gel from 100% whey protein concentrate that had similar hardness to a 100% albumen gel. This was accomplished at both neutral and slightly alkaline pH values. It would be interesting to determine how a 100% whey protein concentrate gel or a blended gel of whey protein concentrate and albumen performs at much higher alkaline pH. One of the practical problems that will then arise may well be flavour. Finding a way to maintain a high pH in such a system without having it taste soapy, as it is wont to do at high alkaline pH values, remains a challenge. But if successful, it will be of immense interest and benefit to the food industry in general. It may then be possible to access all or some of the benefits of a whey protein gel system.

One of the relationships that was not fully quantified was the optimal association between albumen and whey protein concentrate in the gel system and their interaction with total concentration. A 2:1 ratio has

been shown, in the present study, to work best in accentuating the gel hardness. However, there still remains confusion as to what the most favourable ratio is. In a separate study by Ngarize *et al.* (2004) a ratio of 1:2 appeared to produce the hardest gel. If indeed a harder, combination gel can thus be produced, how can it be used to reduce the total dosage of material? For instance, will a different blend produce a gel that can be dosed at less than 16% solids and still produce gel of similar hardness? Is it possible to add the same amount at a slightly higher pH and have the same gel hardness? If this is case, then there are cost savings to be enjoyed because a gel sample can then be produced using fewer raw materials with great benefits to the ecosystems and the environment. This is because the production of both egg and whey protein concentrate require the use of considerable agricultural resources.

The present study has shown that modifying the bound water in the system can alter gel springiness. When the interstices are small, the water in the gel is held tightly and has less of an ability to affect the gel flow properties. This property is important in food systems where the gel is required to maintain its integrity especially in a hot, aqueous and low pH medium. It applies to products such as sausages in a sauce, egg fried rice etc. Quorn products, for instance, typically soften badly when cooked in a *Tikka* sauce. The acidity of the tomato in the sauce is not the causal mechanism. The ability to control the gel characteristics by altering the make up of the gel and the binding of the 'free' water in some way perhaps by the use of hydrocolloids may help to improve the performance of such gel systems. For instance, what happens to springiness of a gel with high free water but which is then bound with a hydrocolloid? Or what happens when the pH at which the gel is formed is far away from the isoelectric pH but on the very low acid (rather than just the alkaline) side of neutral? In reality, it will be interesting to see the changes in the TPA factors at such pH values. As there is a wide range of hydrocolloids and all with different functionalities and *modus operandi*, there is a plethora of opportunities for research in this field.

My experiments have shown that what whey protein concentrate does to an otherwise all-albumen gel is to confer on it a degree of elasticity. Albumen gels tend to be brittle. However, the big problem with whey protein concentrate is the tendency for a gel containing a high level of it is to flow when it has been made at a low pH. No doubt this is due to a lot of the water not being fully bound. Even with the present study, we still do not fully understand how whey protein concentrate contributes to gel structure in conjunction with albumen. At what point is it at its optimum in contributing to elasticity when hot and when cold? What can we as food researchers and chemists do to 'fool' the molecules into behaving atypically even when they are at or near the isoelectric point? It is possible that emulsifiers may be effective in accomplishing this objective. Why? They have hydrophilic and lipophilic moieties that may bind to water molecules at one end and to the protein molecules at the other end, thus forming a bridge that could contribute to gel structure. This experiment was not carried out due to the fact that it would have entailed studying two different materials in the same gel system.

In a pure gel system, the present study has shown that optical properties as measured by Hunter LAB can probably be used as an early warning system to characterize the gel. It may be important to develop complete a tests that attempts to correlate the molecule aggregate size with gel strength and colour. Large aggregates strongly scatter light, thus giving the gel a whiter and more opaque appearance. Fine stranded gels result from conditions that allow the proteins to aggregate in ordered linear filaments. They form a structure that is transparent or translucent and because the strands are so small, they cannot scatter light effectively (Resch *et al.*, 2002).

7.2 Added Materials

Gelation is difficult to define in general terms as gels exhibit diverse microstructures and textural properties. Protein gelation plays a major role in the domestic and industrial production of many foods. Protein is

utilised as a minor or sometimes major, ingredient for the formation of solid visco-elastic foods as well as for improved water absorption, thickening, adhesion, emulsification and foam stabilization. The effect of the protein in defining the characteristics of the food system in which it is used is anything but minor. Due to the fact that many gelling proteins are multi-component in nature, it is not always easy to provide a simple, mechanistic explanation for the method of gelation or to predict precisely and consistently how a gel system is formed from a mixture of raw materials. The multi-component nature of many food systems in which the gelation is required does not help either.

Research into improving these properties or having the knowledge to modify and shift them in specific directions to deliver an exact amount of a required macroscopic property is always going to be of considerable interest in the food industry in general. This is because real food structures are very rarely simple. They are always a combination of several biopolymers with the inclusions of different solutes and solvents. In food, globular proteins are surrounded by a complex mixture of water, sugars, salts, polysaccharides, surfactants and lipids. When gelation occurs, there is almost always competition between the factors favouring gel formation and those favouring phase separation.

It has been shown in the present study that the physico-chemical properties of a heat-induced whey protein concentrate/albumen gel were affected by the actual molecular configuration as well as the environmental conditions. The molecular configuration can be altered by the presence of other micro- and macro-molecules which in turn can either alter or be altered by the environmental conditions such as pH, ionic strength, solvent polarity, morphology, size, surface properties, hydrophobicity, thermo stability, catalytic activity, viscosity, etc.

By combining two different sets of data from the analysis of the gel characteristic, using TPA and stress relaxation, it has been possible to gain more of an insight into the factors that drive the specific qualities of a combined whey protein and albumen gel system.

7.2.1 Pectin

High methoxyl pectin showed an interesting effect on the gel characteristics. It is quite conclusive that the gelation of a mixture of whey and albumen proteins with pectin is achieved via a phase separation between the protein gel and the pectin gel. At concentrations of pectin less than 0.5%, there was the formation of a protein backbone to support the gel. However, at higher concentration of pectin (>0.5%) there was also a phase inversion with the pectin backbone supporting the gel. It is worth exploring in some more detail. For instance, why was the effect of high methoxyl pectin so pronounced when the concentration was in excess of 0.5%? Is it just thermodynamic and molecular incompatibility? Or are there other factors at work? What happens when other types of pectin such as amidated pectin and low methoxyl pectin are used with and without added calcium? How will the pectin affect a binder system with differing ratios of whey protein concentrate to albumen? Turgeon and Beaulieu (2001) stated that phase separation is driven by the behaviour of each biopolymer in solution **prior** to heat-induced gelation and that protein/pectin attractions have to be stronger than protein/protein and pectin/pectin attractions in order to have a compatible and homogenous gel. Yoo *et al.* (2006) also showed that the viscosity of a pectin solution is significantly affected by the presence of different salts at differing concentrations. They concluded that the forces that promote pectin aggregation are complex and involve a combination gel network formation, precipitation and solubilization mechanisms. Protein dissolution tests in the present study suggested that there was stronger affinity of the pectin molecules for each other and for the solvent, in preference to the protein molecules. The pectin molecules interacted via hydrophobic bonds long before the disulphide bonds between the protein molecules had a chance to be established. As pectin is often used to promote succulence in many food products due to their high affinity for water, it is worth exploring in further work ways in which the protein/pectin attraction can be strengthened or conversely, ways in

which the pectin/pectin hydrophobic interaction or the pectin/water affinity can be reduced.

Nevertheless, the phase separation between the protein molecules and the pectin molecules can be used to good advantage. Pectin can be used to alter the brittleness of a protein gel or at the other extreme; protein can be used to increase the elasticity of a pectin gel. The interplay between the two may also be useful in molecule encapsulation in which the molecules or particles of other material and 'embedded' in one phase which can then be held in another composite e.g. heat labile flavours, vitamins, nutraceutical molecules etc.

7.2.2 Inulin

Inulin is also another ingredient perhaps worthy of further investigation. It is unknown how inulin reacts to low pH conditions when in a gel system. However, it is known that inulin can be hydrolysed by the conditions common in gel formation namely i.e. heating temperatures greater than 80°C and a pH of about 6 – 8 (Kim *et al.*, 2001).

7.2.3 Starch

Starches are important hydrocolloids. The present study showed how the rice starch, high amylopectin starch and high amylose starch increased the hardness of an albumen/whey protein concentrate gel. Protein dissolution test results suggested that for the high amylose starch, there was the formation of an interpenetrating gel between the starch molecules and the protein molecules. The CLSM micrographs showed that a fine-stranded interpenetrating gel was formed at pH 5 but changed to a phase-separated or particulate gel at pH 6. Results also showed how the starches reduced the expressible water especially at pH 5.0.

As a proposal for further studies, an aspect for further research will be to determine how the water is bound in the gel. What happens as the starches start to age and retrograde? Has the optimum concentration really been determined via these experiments? What has

the starch actually done to the water in the context of a protein gel? It is known that water serves as a plasticizer and thus influences the molecular mobility of the starch molecules (Choi and Kerr, 2003). Have the starch granules fully gelled and lost their amorphousness or are they exerting their influence on water by another mechanism? Nuclear Magnetic Resonance (NMR) may help answer some of these questions. Hinrichs *et al.* (2003) and (Choi and Kerr, 2003) describe a method for using NMR to measure and characterize the structure and water-holding capacity of hydrocolloid gels. They concluded that this method offers molecular information about the structure of gels and helps to quantify the influence of ingredients and processing on the gel properties.

7.2.4 Soya Protein Isolate

Soya isolate is another ingredient whose performance in the present study raises a few issues. As a globular protein, it is expected to unfold and eventually form a gel with the application of heat in an aqueous environment. However, it does not always gel fully and has been reported to form an aggregate/coagulum (Comfort and Howell, 2002). An exciting thought is to determine, by experimentation, the optimum conditions that will produce a gel from the combination of egg albumen, whey protein concentrate and soy isolate. It has been suggested by soya protein isolate manufacturers (personal communication) that under ambient conditions, it can take up to two hours under conditions of low to medium shear, for soy protein isolate to become fully hydrated. It is not known at present how significant this is in practical terms. No attempts were made in the present study to incorporate this knowledge into the experiments. If it is indeed confirmed that the soya protein was somehow not fully hydrated prior to heat-induced gelation with the albumen and whey protein concentrate, then it is quite possible that its functionality and effect on the gel properties were not fully realised. It would be of interest to repeat the study with soya protein isolate and determine by various means what effect the degree of hydration of the soya isolate has on the gel properties.

7.2.5 Salts

There were a few instances during the present study whereby the effects of the salt addition to the binder system – NaCl and $CaCl_2$ were reviewed. It was speculated that one of the reasons for differences in the results from many disparate studies was because not all researchers carried out the dialysis. Indeed, no dialysis was carried out in the present study. In future research, it will be of scientific interest to determine precisely how to control the gel qualities. In addition many industrial food production recipes call for a reduction in the use of NaCl due to its implication in high blood pressure and heart problems. There have been very few if any studies on the effects of other salt replacements such as potassium chloride and glutamic acid on the protein gel characteristics.

7.2.6 Sugars

Reducing sugars are known to participate in a Maillard reaction with amino acids. This results in crosslinking of the amino acid molecules leading to gelation. It has often been speculated that it might be possible to use the Maillard reaction to promote protein gelation. The results observed in the fracture strain of the gels containing lactose may be due to a Maillard type gelation, but this cannot be confirmed at this stage and is worthy of further investigation. Previous experiments have suggested that when globular proteins are retorted in the presence of a reducing sugar, gelation occurred at low protein concentrations (Hill *et al.*, 1992).

7.3 Further work

In addition to suggestions for further work mentioned in the previous section, there are other areas that warrant further attention.

7.3.1 Fresh egg and fresh whey

All the experiments described in the present study were carried out using dehydrated egg and whey protein concentrate. Pasteurised egg albumen powders generally produce harder gels than the non-pasteurised versions due to increased surface hydrophobicity as a result of the heat of pasteurization (Delben and Stefancich, 1998). However, there is immense cost associated with drying and then pasteurising the egg white. If it is done solely for the purpose of producing a firmer, harder gel, then it must be worth the while to research ways in which liquid, unprocessed egg albumen can be made to form harder gels. Besides, the process of drying, no matter how carefully it is controlled, will almost certainly produce some damage to some of the protein, resulting in a loss of or reduction in functionality. The use of liquid versions of both albumen and the whey protein concentrate to test the effects of selected added materials will be of advantage in the part of the food industry where the use of liquid egg is still the norm e.g. sugared egg for use in the cake industry.

7.3.2 Whey protein fractions and avian egg albumen

The diverse fractions of whey protein have different effects on each other. For instance, it was reported that α-lactalbumin affects the gelation characteristics of β-lactoglobulin and that the denaturation temperature for β-lactoglobulin is reduced in the presence of α-lactalbumin. It was also suggested that the unfolding of α-lactalbumin on denaturation might initiate cross–linking interactions with exposed sites on the β-lactoglobulin molecule, thus starting a chain reaction in speeding up the unfolding of the β-lactoglobulin molecule (Boye and Alli, 2000). What is not fully understood at the present time is whether there is such an effect with egg albumen and fractions of whey protein. Suggestions for further work to examine the effect of the following:

I. albumen and pure α-lactalbumin,
II. albumen and pure β-lactoglobulin and
III. albumen and pure bovine serum albumin.

There may also be different combinations of the fractions that produce different types of gels.

7.3.3 Avian egg albumen fractions and whey protein concentrate

Fractions of albumen should be tested against a nominal whey protein concentrate. There are two ways in which this may demonstrate benefits. Whey protein concentrate is normally either made directly by using acid to precipitate the whey from milk, or it is made available as a by-product of cheese production. As a waste stream, the liquid whey needs to have significant amounts of water removed in order to yield a concentrate. If fractions of egg are made available, they can be added directly to the whey stream, thereby obviating the requirement to concentrate and then dry the whey protein. The use of egg albumen fractions such as ovalbumin in conjunction with other materials may also produce gels of different characteristics and is worthy of further investigation.

7.3.4 Transglutaminase

Transglutaminases are a family of microbial enzymes that catalyze the formation of a covalent bond between a free amine group (e.g., protein- or peptide-bound lysine) and the γ-carboxamide group of protein- or peptide bound glutamine. Transglutaminase has been used in applications such as binding small chunks of meats into a big one ("portion control"), such as in sausages, hot dogs, recombined meat, improving the texture of low-grade meat such as so-called pale, soft, and exudative meat, making milk and yoghurts creamier and making noodles firmer. It has also been used to bind GMP in milk products (Tolkach and Kulozik, 2005). Although it was shown in the study that native whey proteins are not a good substrate for transglutaminase, no work has been done with albumen to determine whether partially denatured albumen, may show some effect on he resulting gel. This can form the basis for further work

7.3.5 Multicomponent gels

Many of the tests carried out in the present study were done with the materials added one at a time. The experiments were purposely designed in this manner in order to quantify the effect of such materials in a pure gel system. In reality, as foods contain a wide variety of different components, it is important to enumerate the effect of combinations of the materials by means of a larger, all-encompassing study. Response surface methodology is a good way to study such effects in a combined whey and albumen gel system. There is also more likelihood for the formation of mixed systems under such conditions where the materials exert differing effects on the albumen and whey protein gel structure. If there are any further synergistic or antagonistic behaviour between the groups of materials, these can be elucidated by means of such a study.

The speed and order of gelation could also be important. For example, Ziegler and Rizvi (1989) showed that in a mixed gel system, it is the network that forms first that usually exhibits the greater degree of continuity even if it is the minor component by weight. The primary network is also the one that tends to carry the most load and thus determines the mechanical properties of the mixed gel. In another study, Gonçalves *et al.*, (2004) used cassia gum to modify the heat-induced gelation of whey protein isolate in a system that would normally have exhibited phase separation. Prior to this time, a different study had suggested that locust bean gum hampered protein/protein interactions in whey protein gel and that the chains of the gum molecules were accommodated in the continuous protein network causing a 'micro-phase separation' rather than a full phase separation. The degree of the micro-phase separation was shown to be dependent on pH (Tavares and Lopes da Silva, 2003). This proves that it is possible to retard the rate or extent of phase separation. There is a basis for further research here. Do any of the added materials have a higher affinity for the whey proteins than the albumen proteins? Or do they affect the solvent in such a way that they alter the affinity of the

protein molecules for the solvent? If any of these apply, can they be modified such that preferential gelation of a selected molecule (or group of molecules) can be induced? This will be of considerable interest in creating novel products within the food industry.

7.3.6 Type of acid

Acidification of all the gel samples used in the present study, were carried out with hydrochloric acid. Previous protein gelation studies have used other acids such as lactic acid, phosphoric acid, citric acid, tartaric acid, acetic acid and glucono delta lactone to lower the pH (Jelen and Bucheim, 1984; Venugopal *et al.,* 1994; Boye *et al.,* 1997; Ju and Kilara, 1998). Data suggests that the use of different acidulants produced gels with a range of functionalities. This is due to the fact that the introduction of different ions into a solvent can cause alterations in the solvent, which in turn can affect the solubility, unfolding and aggregation of the protein molecule (Damodaran and Kinsella, 1982). The Hofmeister series also postulates that different anions have different effects on the structure of protein and other macromolecules.

As many foods use one or more of the different acids during manufacture, it is known how many of the observations in this study will still hold true if an acid other than hydrochloric acid was used. For instance Resch *et al.* (2005) reported that there were differences in the fracture strain values of a whey protein gels made with lactic acid, citric acid and hydrochloric acid. More common food acids should be tested for their effect on the properties of the resulting gel.

7.3.7 Effect of reducing sugars

There is indication that more reactive reducing sugars may lead to a stronger gel when reacted with globular proteins although they are also likely to form a darker product (Mitchell and Hill, 1995). In a more recent study, Kwan and Easa (2003) used glucose as a Maillard active molecule in making retort tofu and reported that the retorted tofu was firmer than the control sample. However, a few other studies suggest

that glucose has very little effect on Maillard crosslinking (Mitchell and Hill, 1995; Graham, 1996).

In the present study, only dextrose and lactose were used as sugar sources. It will be interesting to determine the effect of other sugars especially the pentoses such as ribose and xylose in their ability to act as a precursor for Maillard crosslinking.

7.3.8 Confocal Laser Scanning Microscopy

One of the constraints in the present study has been the difficulty within the time frame of the study to fully define and encapsulate the differences between a particulate gel and a phase-separated gel. Even with the so-called fine-stranded gels, resolution was only achieved up to about 150–300 microns. There are a few studies that have reported resolution as low as 2-5 microns. It is thought that at this level of resolution, it should be possible to show the difference between a phase-separated and particulate gel.

Furthermore, as many foods consist of a lot more than just two components, differential and multiple staining of a gel sample for CLSM will be necessary to further elucidate the relationship between micro and macro molecules and their effect on protein gel properties. Arltoft *et al.* (2007a) reported some success with differential staining of pectin and carageenan in a dairy dessert and were able to show clear evidence of a coupled gel between the pectin and carageenan in a continuous casein matrix. For instance, in the protein/pectin gels reported in the present study, it would have been useful to be able to stain the pectin with a fluorescent marker in order to be able to monitor its position within the gel structure and its affinity for water. Staining of the pectin phase is difficult but there are ongoing studies within this field to define an effective pectin staining procedure (personal communication). Protein molecules can easily be labelled because the label molecules tend to be hydrophobic and will therefore prefer to sit in the protein rich areas. Pectin is hydrophilic and therefore has no affinity for fluorescent label molecules, which tend to be more hydrophobic. Pectin and most other polysaccharides have to be labelled by binding

the label molecules covalently (Tromp *et al.,* 2004). The binding takes place by a chemical reaction of the reactive label with the hydroxyl groups on the pectin chain. The danger is that sometimes the extra charges introduced to the pectin chain change the properties of the pectin. In the experiment by Arltoft *et al.* (2007a) the pectin was stained with an anti-pectin antibody *JIM5*. Further tests have also been carried out by Arloft *et al.* (2007b) using monoclonal pectin antibody *JIM7*. If available, this antibody may find widespread use in pectin staining for CLSM studies.

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APPENDIX 1

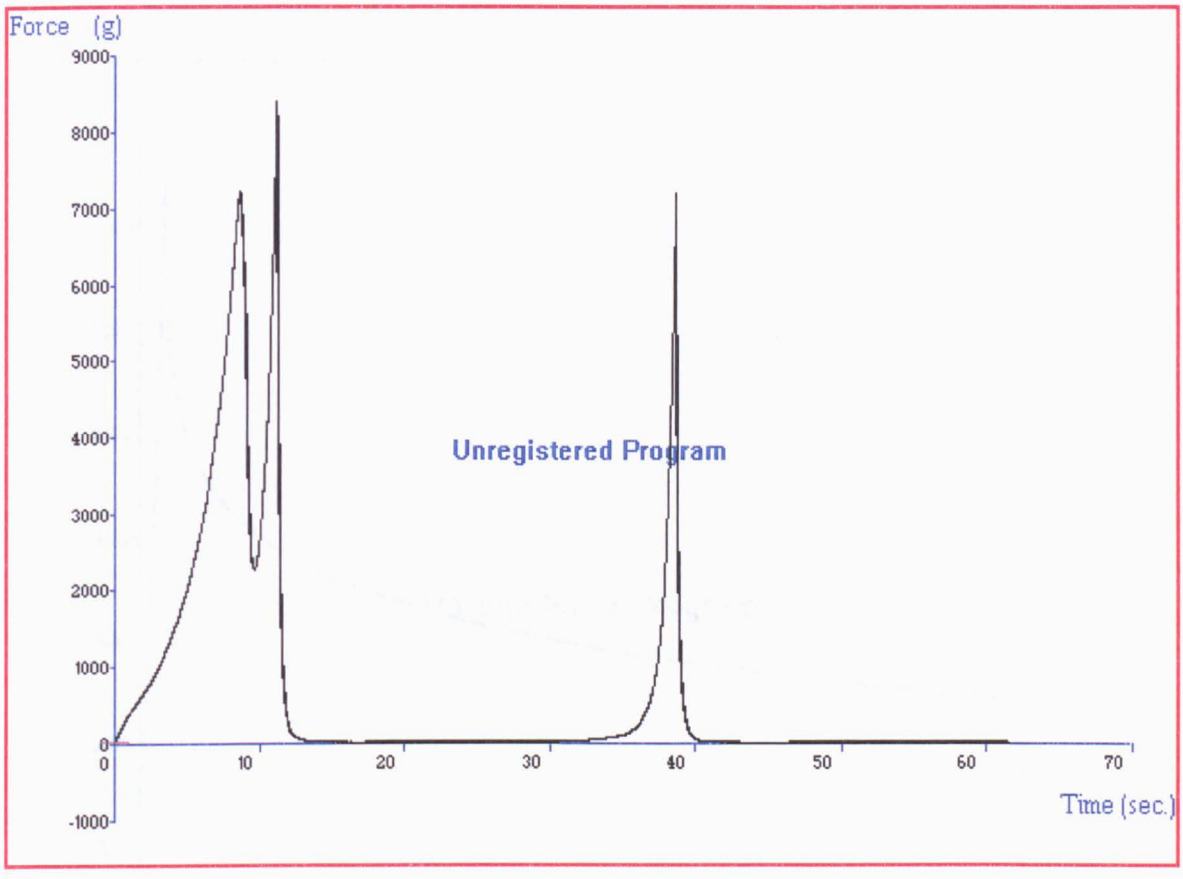
RAW DATA PARAMETERS FOR LACTOSE GEL

EXPERIMENT NUMBER	FACTOR 1 pH	FACTOR 2 Albumen : Whey	FACTOR 3 Lactose (%)	a-VALUE	b-VALUE (1/s)	WATER UPTAKE (%)	WATER LOSS (%)	HARDNESS (g)	SPRINGINESS	COHESIVENESS	GUMMINESS	CHEWINESS	RESILIENCE	INITIAL MODULUS (N/m^2)	FRACTURE STRAIN (%)	FRACTURE STRESS (kN/m^2)
1	5	1.5/1	0	0.753	3.33	3.39	5.20	8693.03	0.53	0.21	1818.07	1032.20	0.07	1582.12	63.66	242.75
2	5	1.5/1	0	0.753	3.34	3.46	5.02	8987.56	0.52	0.21	1816.56	965.17	0.06	1541.64	63.13	232.82
3	5	1.5/1	0	0.753	3.34	3.36	4.91	8855.89	0.52	0.22	1793.02	923.20	0.06	1591.62	63.06	253.08
4	6	1.5/1	0	0.647	3.38	2.36	1.61	8967.80	0.72	0.19	1721.14	1245.77	0.06	1533.14	16.98	313.18
5	6	1.5/1	0	0.646	3.37	2.01	1.33	11560.74	0.70	0.23	2627.40	1496.56	0.08	1515.28	70.72	306.22
6	6	1.5/1	0	0.644	3.37	1.89	1.45	11243.86	0.70	0.22	2126.35	1620.98	0.08	1465.13	68.04	273.14
7	5	2/1	0	0.735	3.31	3.12	3.70	9422.01	0.60	0.21	1942.70	1155.61	0.06	1761.79	63.26	282.96
8	5	2/1	0	0.737	3.31	3.02	3.68	10023.45	0.59	0.21	2053.01	997.78	0.06	1851.80	63.02	262.99
9	5	2/1	0	0.734	3.30	3.16	4.17	9571.29	0.49	0.20	1942.07	959.82	0.06	1795.84	63.39	272.94
10	6	2/1	0	0.637	3.26	1.88	0.90	9689.08	0.72	0.24	2376.69	1759.03	0.07	1595.55	66.12	263.28
11	6	2/1	0	0.638	3.30	2.03	1.13	10148.34	0.74	0.25	2152.39	1682.90	0.07	1521.61	65.12	273.15
12	6	2/1	0	0.638	3.24	1.89	0.91	9475.20	0.73	0.23	2172.01	1583.42	0.07	1511.56	65.10	253.12
13	5	2.5/1	0	0.742	3.44	4.03	3.79	11124.11	0.55	0.22	2237.19	1234.83	0.06	1948.02	67.67	353.36
14	5	2.5/1	0	0.744	3.42	3.98	3.73	12057.54	0.56	0.21	2069.82	1265.25	0.06	1937.08	67.64	353.41
15	5	2.5/1	0	0.739	3.43	3.89	3.84	11672.59	0.58	0.22	2116.63	1176.74	0.06	1887.26	66.56	343.33
16	6	2.5/1	0	0.629	3.26	0.89	0.92	10221.89	0.73	0.21	2176.05	1590.48	0.06	1685.41	71.17	363.51
17	6	2.5/1	0	0.629	3.23	0.74	1.11	9657.85	0.71	0.21	2221.08	1432.27	0.06	1715.41	71.80	373.51
18	6	2.5/1	0	0.626	3.26	0.91	0.92	9402.47	0.72	0.21	2114.13	1433.97	0.06	1675.42	71.74	363.27
19	5	1.5/1	0.5	0.738	3.39	3.52	4.76	9649.17	0.57	0.21	1972.12	1117.12	0.06	1625.73	64.01	262.96
20	5	1.5/1	0.5	0.736	3.35	3.14	4.89	9040.45	0.61	0.20	1787.52	1124.05	0.05	1635.88	64.04	252.99
21	5	1.5/1	0.5	0.739	3.35	3.17	4.86	8726.60	0.59	0.20	1858.81	1130.48	0.06	1545.86	63.61	252.85
22	6	1.5/1	0.5	0.640	3.25	2.12	1.23	9189.47	0.80	0.21	1941.13	1409.06	0.07	1556.12	66.44	272.95
23	6	1.5/1	0.5	0.641	3.26	2.10	1.21	8535.45	0.72	0.20	1991.15	1598.41	0.07	1535.56	65.48	273.07
24	6	1.5/1	0.5	0.640	3.22	2.00	1.20	8872.21	0.82	0.20	1916.40	1575.86	0.07	1543.25	66.00	273.26
25	5	2/1	0.5	0.739	3.26	3.94	5.08	10191.15	0.50	0.20	1824.73	1242.50	0.07	1839.36	63.91	272.79
26	5	2/1	0.5	0.740	3.25	3.93	4.97	9153.65	0.51	0.22	2294.02	1100.64	0.07	1778.60	63.33	272.90
27	5	2/1	0.5	0.740	3.28	3.84	5.04	9239.68	0.53	0.21	1968.78	1206.44	0.07	1819.66	64.57	262.77
28	6	2/1	0.5	0.633	3.18	2.11	1.23	9060.50	0.77	0.24	1942.52	1396.27	0.07	1567.58	67.38	313.54
29	6	2/1	0.5	0.630	3.19	1.90	1.20	9369.28	0.74	0.22	2058.24	1526.08	0.07	1547.10	67.22	313.55
30	6	2/1	0.5	0.629	3.17	2.23	1.28	9330.01	0.74	0.22	1919.69	1414.19	0.07	1457.36	68.80	313.48
31	5	2.5/1	0.5	0.750	3.37	4.09	4.24	10333.30	0.51	0.22	2310.89	1297.30	0.06	1874.44	66.81	333.21
32	5	2.5/1	0.5	0.748	3.41	4.23	4.20	10304.08	0.55	0.22	2345.08	1275.35	0.06	1864.89	66.65	329.37
33	5	2.5/1	0.5	0.748	3.39	4.32	4.11	10301.22	0.51	0.23	2313.30	1263.75	0.06	1777.15	66.72	323.31
34	6	2.5/1	0.5	0.627	3.20	1.63	1.34	9557.05	0.72	0.21	1838.25	1228.46	0.06	1715.05	70.15	363.41
35	6	2.5/1	0.5	0.626	3.22	1.67	1.23	9524.95	0.74	0.20	1771.12	1200.82	0.06	1630.48	69.69	373.40
36	6	2.5/1	0.5	0.627	3.22	1.68	1.31	9360.23	0.75	0.20	1846.89	1212.28	0.06	1646.17	70.15	373.04
37	5	1.5/1	1	0.747	3.26	3.80	4.90	9169.71	0.59	0.22	2213.67	1495.78	0.07	1534.06	63.69	242.24
38	5	1.5/1	1	0.749	3.30	3.68	5.02	9440.97	0.58	0.23	2335.39	1352.89	0.07	1595.66	63.10	242.33
39	5	1.5/1	1	0.749	3.31	3.71	5.06	9773.08	0.59	0.22	2568.66	1552.37	0.07	1555.27	63.57	241.44
40	6	1.5/1	1	0.650	3.46	1.93	1.22	9447.50	0.79	0.22	2081.89	1642.02	0.07	1695.84	65.76	293.00
41	6	1.5/1	1	0.642	3.38	2.09	1.05	8914.54	0.80	0.23	2003.52	1606.55	0.07	1656.25	66.05	292.90
42	6	1.5/1	1	0.642	3.39	1.67	1.08	9401.95	0.82	0.23	2075.10	1617.38	0.07	1656.72	65.12	293.00
43	5	2/1	1	0.740	3.27	3.80	4.86	9424.89	0.56	0.22	2445.83	1034.69	0.06	1764.42	64.31	284.13
44	5	2/1	1	0.742	3.26	3.57	4.95	10269.00	0.54	0.22	2036.90	1132.01	0.06	1745.90	64.16	272.89
45	5	2/1	1	0.742	3.25	3.85	4.76	10016.15	0.53	0.23	2359.85	1089.78	0.06	1696.21	64.16	278.88

EXPERIMENT NUMBER	FACTOR 1 pH	FACTOR 2 Albumen : Whey	FACTOR 3 Lactose (%)	a-VALUE	b-VALUE (1/s)	WATER UPTAKE (%)	WATER LOSS (%)	HARDNESS (g)	SPRINGINESS	COHESIVENESS	GUMMINESS	CHEWINESS	RESILIENCE	INITIAL MODULUS (N/m^2)	FRACTURE STRAIN (%)	FRACTURE STRESS (kN/m^2)
46	6	2/1	1	0.634	3.29	1.75	0.89	9830.66	0.74	0.22	2052.35	1448.77	0.07	1724.41	68.22	329.66
47	6	2/1	1	0.635	3.29	1.63	0.93	9770.12	0.71	0.22	2122.17	1536.90	0.07	1745.03	68.49	315.18
48	6	2/1	1	0.633	3.27	1.65	1.07	9781.95	0.75	0.22	2041.15	1583.14	0.07	1745.41	68.37	333.20
49	5	2.5/1	1	0.747	3.45	3.75	3.99	11418.88	0.60	0.23	2630.05	1521.74	0.06	1798.55	67.02	321.57
50	5	2.5/1	1	0.749	3.46	3.74	3.99	11013.60	0.60	0.23	2516.43	1501.81	0.06	1778.26	67.26	319.17
51	5	2.5/1	1	0.747	3.48	3.52	4.10	11044.39	0.59	0.23	2522.57	1492.70	0.06	1748.00	66.60	326.05
52	6	2.5/1	1	0.626	3.24	2.22	0.87	9787.20	0.71	0.20	1935.89	1235.84	0.06	1675.27	71.62	435.32
53	6	2.5/1	1	0.628	3.22	2.16	0.87	9650.36	0.70	0.20	1907.27	1202.20	0.06	1695.02	71.19	393.57
54	6	2.5/1	1	0.627	3.26	2.25	0.91	9758.81	0.69	0.20	1768.81	1152.75	0.06	1655.13	71.54	343.60
55	5	1.5/1	1.5	0.752	3.33	3.94	4.67	7908.83	0.52	0.22	1795.69	930.95	0.06	1580.94	65.27	286.29
56	5	1.5/1	1.5	0.752	3.35	3.91	4.59	8512.59	0.52	0.21	1851.97	977.28	0.06	1628.65	65.11	272.59
57	5	1.5/1	1.5	0.751	3.38	3.88	4.67	8790.38	0.48	0.22	1864.30	971.06	0.06	1545.96	65.77	272.53
58	6	1.5/1	1.5	0.654	3.31	2.18	1.23	9479.33	0.83	0.21	1955.16	1615.55	0.07	1515.84	67.54	293.40
59	6	1.5/1	1.5	0.656	3.37	2.14	1.22	9471.38	0.80	0.23	2220.71	1771.04	0.07	1458.89	66.18	273.26
60	6	1.5/1	1.5	0.654	3.32	1.96	1.40	8926.55	0.66	0.23	2078.20	1372.24	0.07	1588.94	65.25	253.43
61	5	2/1	1.5	0.744	3.33	3.19	4.55	11714.21	0.65	0.23	2393.91	1549.50	0.06	1886.14	63.59	283.15
62	5	2/1	1.5	0.742	3.32	3.49	4.74	11411.19	0.60	0.23	2462.81	1442.93	0.07	1776.31	63.02	293.16
63	5	2/1	1.5	0.743	3.31	3.28	4.70	10526.42	0.63	0.23	2255.70	1559.49	0.07	1777.39	64.88	293.27
64	6	2/1	1.5	0.647	3.23	1.97	1.18	8732.70	0.73	0.20	1678.39	1164.43	0.07	1516.83	68.42	323.55
65	6	2/1	1.5	0.647	3.22	2.03	1.28	8530.41	0.68	0.21	1797.42	1219.83	0.07	1476.46	69.32	313.53
66	6	2/1	1.5	0.641	3.18	2.02	1.11	8051.53	0.73	0.21	1507.19	1217.40	0.07	1552.85	68.61	313.52
67	5	2.5/1	1.5	0.741	3.34	3.23	3.66	11559.88	0.51	0.25	2849.32	1465.80	0.06	1756.67	66.56	323.49
68	5	2.5/1	1.5	0.742	3.34	2.89	3.94	11853.07	0.52	0.22	2817.98	1497.83	0.06	1753.17	67.56	343.33
69	5	2.5/1	1.5	0.740	3.39	3.50	3.71	11660.37	0.53	0.23	2689.23	1539.26	0.06	1758.10	67.43	313.45
70	6	2.5/1	1.5	0.626	3.17	2.11	0.91	8110.89	0.68	0.18	1248.29	977.11	0.05	1638.10	74.94	333.56
71	6	2.5/1	1.5	0.627	3.20	2.15	0.88	7163.54	0.73	0.17	1134.06	773.84	0.05	1546.79	73.02	343.57
72	6	2.5/1	1.5	0.625	3.22	2.12	0.89	8049.46	0.69	0.17	1204.78	875.09	0.05	1645.55	72.67	351.48
73	5	1.5/1	2	0.751	3.27	3.21	4.93	9426.62	0.67	0.23	2038.56	1093.44	0.06	1581.26	65.26	242.70
74	5	1.5/1	2	0.751	3.29	3.18	4.96	9123.03	0.66	0.22	1864.08	1054.15	0.06	1577.72	64.38	252.67
75	5	1.5/1	2	0.751	3.28	3.25	5.12	8631.19	0.62	0.22	1937.91	1038.45	0.06	1578.50	64.30	252.69
76	6	1.5/1	2	0.643	3.20	2.22	1.41	7839.32	0.75	0.24	2045.33	1587.58	0.07	1664.10	64.53	252.90
77	6	1.5/1	2	0.641	3.28	2.31	1.37	8944.94	0.77	0.23	2390.26	1622.87	0.08	1574.21	64.32	252.89
78	6	1.5/1	2	0.641	3.21	2.11	1.32	8397.84	0.78	0.24	1691.61	1773.21	0.08	1634.18	66.15	252.89
79	5	2/1	2	0.753	3.35	3.42	4.19	9252.06	0.54	0.21	1909.94	1139.02	0.06	1636.72	65.18	293.04
80	5	2/1	2	0.750	3.34	3.53	4.41	9035.12	0.55	0.22	2045.21	1113.92	0.06	1636.22	65.29	283.04
81	5	2/1	2	0.748	3.35	3.32	4.38	9162.46	0.60	0.22	1828.76	1144.25	0.06	1675.45	65.13	283.00
82	6	2/1	2	0.635	3.24	1.35	1.22	9212.07	0.72	0.19	1768.40	1271.89	0.06	1496.10	69.67	333.41
83	6	2/1	2	0.634	3.21	1.37	1.22	8883.60	0.73	0.21	1941.11	1226.51	0.06	1556.33	68.00	323.56
84	6	2/1	2	0.633	3.19	1.57	1.25	9101.90	0.71	0.20	1855.02	1317.72	0.06	1525.37	69.72	323.57
85	5	2.5/1	2	0.744	3.30	3.99	3.99	11043.61	0.54	0.22	2375.86	1292.95	0.06	1726.27	65.81	297.09
86	5	2.5/1	2	0.743	3.34	3.78	4.22	11083.12	0.55	0.23	2495.93	1367.16	0.07	1752.66	65.49	331.27
87	5	2.5/1	2	0.744	3.34	3.85	4.15	11298.89	0.53	0.21	2389.80	1245.33	0.06	1773.86	65.52	301.63
88	6	2.5/1	2	0.630	3.17	1.96	1.11	8195.40	0.70	0.20	1999.27	1297.61	0.06	1625.00	72.30	423.31
89	6	2.5/1	2	0.632	3.22	1.80	1.12	8973.73	0.73	0.20	1594.86	1119.45	0.06	1747.06	73.82	423.28
90	6	2.5/1	2	0.631	3.15	1.75	1.05	7804.81	0.68	0.21	1897.92	1375.40	0.06	1649.24	72.74	413.30

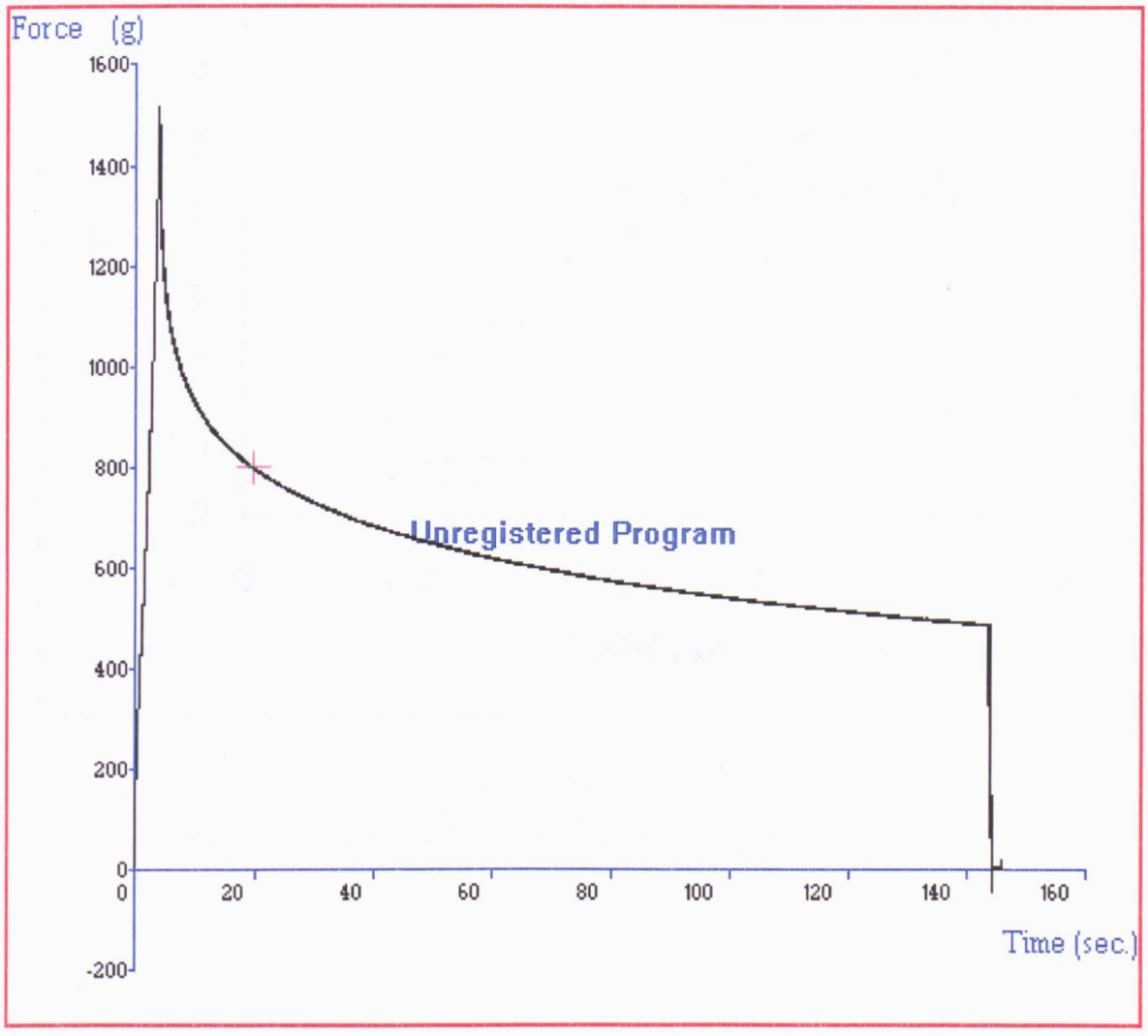
APPENDIX 2

PICTORIAL ILLUSTRATION OF TYPICAL TPA PLOT FOR LACTOSE GEL



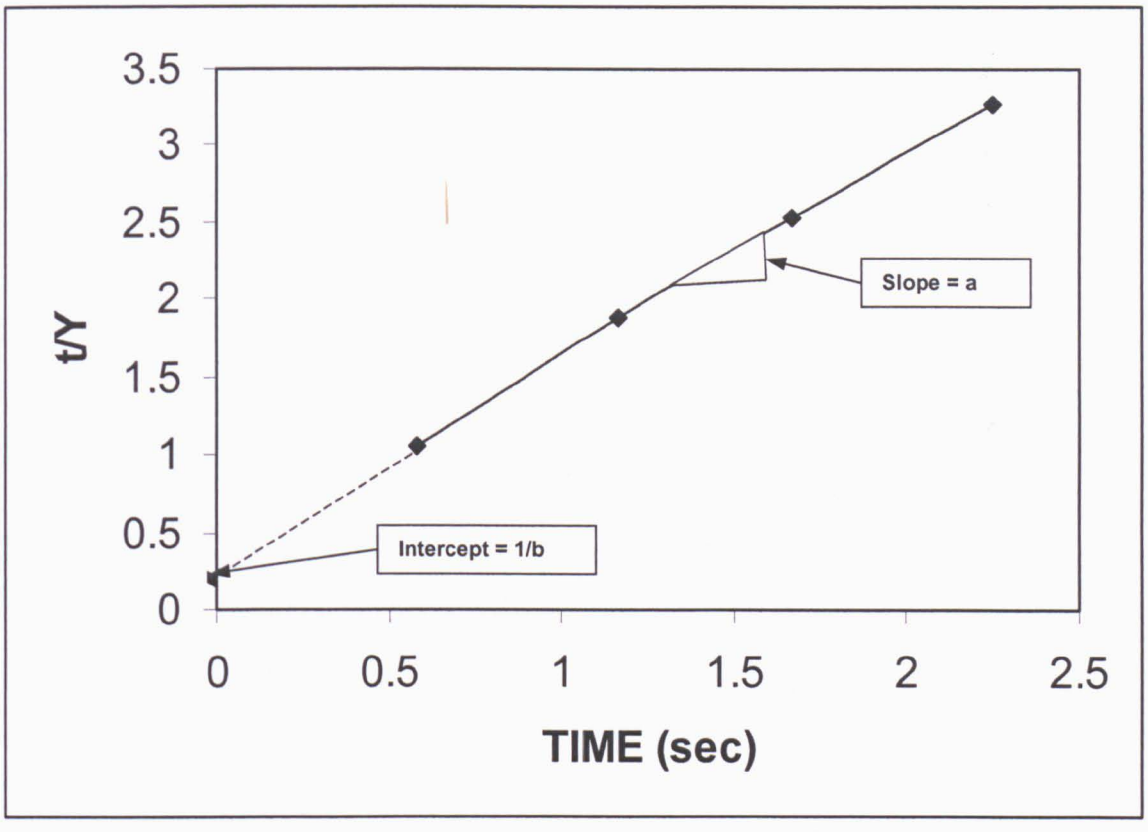
APPENDIX 3A

PICTORIAL ILLUSTRATION OF TYPICAL STRESS RELAXATION PLOT FOR LACTOSE GEL



APPENDIX 3B

PICTORIAL ILLUSTRATION OF LINEARIZED STRESS RELAXATION PLOT FOR LACTOSE GEL (converted via Excel Spreadsheet)



$$\frac{t}{Y(t)} = \frac{F_o t}{F_o - F(t)}$$

Where F_o = force at t = o and F(t) is force at time (t) during compression cycle