THE RELATIONSHIP BETWEEN THE GELATION PROPERTIES OF SOME MIXED PROTEIN-POLYSACCHARIDES AND THEIR THERMODYNAMIC COMPATIBILITY IN AN AQUEOUS MEDIUM

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- Nursing 0569
- Nutrition 0570
- Obstetrics and Gynecology 0380
- Occupational Health and Therapy 0354
- Ophthalmology 0381
- Pathology 0571
- Pharmacology 0419
- Pharmacy 0572
- Physical Therapy 0382
- Public Health 0573
- Radiology 0574
- Recreation 0575
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- Toxicology 0383

Home Economics 0386

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BY

RONGXUAN CAI

A Thesis/Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements for the degree of

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ABSTRACT

The relationship between gelation properties and thermodynamic compatibility has been established experimentally for BSA- and canola protein-polysaccharide systems. Gelation properties were assessed by means of a small amplitude oscillatory rheological test using a Bohlin rheometer, whereas the thermodynamic compatibility was assessed using turbidimetric analysis, gel filtration and visual assessment. Protein-anionic polysaccharide systems were found to be compatible while neutral polysaccharide systems were incompatible in the concentration and pH regions studied. Two possible binding models, distinguished by particle binding model and chain segment binding model were found in the compatible systems. The particle binding model was found in both BSA and canola 12S globulin systems, while the segment binding model was only found in BSA systems. In compatible systems, gelation properties largely depended on the binding model and thermodynamic conditions. Significantly positive effects were found for BSA-anionic polysaccharide systems if the condition favoured a chain segment binding model. However, anionic polysaccharides were found to be detrimental to the gelation properties of canola protein systems since the chain segment binding model seemed unlikely to occur in those systems. On the other hand, incompatible systems generally exhibited the combined properties of the individual components. While methyl cellulose was found to be detrimental to BSA systems at low protein concentration, it had a beneficial synergistic effect on the gelation properties of canola protein isolate at low protein concentrations. Guar gum with low protein concentrations was also found to have beneficial effect on the canola protein gelation.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

The interactions between protein and polysaccharide are of key importance in determining food texture and have been the subject of many investigations (Ledward, 1994; Tolstoguzov, 1986; Ziegler and Foegeding, 1990). One physicochemical aspect of these interactions is the thermodynamic compatibility of the biopolymers, which has been described as a common phenomenon in multicomponent food systems. Phase diagrams have been used in interpreting the compatibility based on phase separation corresponding to different ratios and concentrations of the biopolymers for casein-soybean globulins system (Polyakov et al., 1980) and for other protein-polysaccharide systems such as soybean globulin-sodium alginate, -dextran system (Tolstoguzov, 1989; Tolstoguzov et al., 1985). Tolstoguzov (1991) further demonstrated that a compatible system may result from the formation of a complex and an incompatible one due to inhibition of complex formation. Thermodynamic compatibility described so far was based on the phase separation behaviours of biopolymers as a function of concentration and dynamic parameters such as pH, ionic strength and temperature (Tolstoguzov, 1991; Tolstoguzov et al., 1985). As a result, a molecular binding model is necessary to provide the detailed information concerning molecular behaviours of the biopolymers in terms of compatibility. On this basis, gel filtration with the assistance of other techniques including turbidimetric analysis and visual assessment were employed in this research to assess the molecular behaviour in selected pH ranges. The combination of these techniques provides information concerning thermodynamic compatibility on a molecular basis, to supplement literature reports based on the phase diagrams (Polyakov et al.,

1980; Tolstoguzov, 1988; 1989; Tolstoguzov et al., 1985; Tolstoguzov and Braudo, 1983).

Among functional properties of the resulting interacting biopolymer systems, gelation properties have received great attention (Bernal et al., 1987; Ustunol et al., 1992; Xiong and Blanchard, 1993). Forces responsible for the interaction of mixtures and the resulting gels were attributed to hydrogen, hydrophobic and covalent interactions and, in particular, electrostatic bonds. For gels formed in different environments, differences in solubility and gelation properties have been demonstrated resulting in the identification of two different types of association between proteins and polysaccharides: the titration complex (T-complex) and the mixture complex (M-complex) (Tolstoguzov, 1986; Tolstoguzov et al.,1985). Higher solubility and enhanced gelation properties for the T-complex was suggested to be attributed to the different binding mechanisms, where T-complexes are protein particles with a single charged polysaccharide chain, resulting in monomatrix, while M-complexes represented entangled networks of polysaccharide chain cross-linked by the protein, resulting in polymatrix complexes (Tolstoguzov, 1986). However, little comparison in terms of gelation properties has been made for differently charged systems resulting from pH adjustment or for different polysaccharide systems, though these are important as part of fundamental research in this area.

Furthermore, most of the previous works concentrated either on the compatibility or on the rheological properties and seldom constructed a relationship between the two, though the relationship may reveal the general roles of the interaction between the biopolymers and these roles' contribution to their rheological properties, and thus, may provide valuable information on food formulation and processing.

The present work detailed the compatibility of these systems at a molecular level and used these data to infer a general mechanism related to the observed rheological

properties. BSA was used in this study to assess a general protein performance in the environments used and to serve as a guide for further investigation of canola protein. Sodium alginate and pectin were chosen to represent the anionic polysaccharides, while methyl cellulose and guar gum were neutral polysaccharides.

The objectives of this research were to reveal the interaction behaviours of some protein (including BSA and canola protein) and polysaccharide mixtures through the investigation of thermodynamic compatibility, to demonstrate the impact of these interactions on protein gelation properties and to infer a mechanism concerning how the gelation properties relate to the interaction among components.

II. LITERATURE REVIEW

A. Chemistry and Gelling Mechanisms of Typical Polysaccharides

Typical gelling polysaccharides include carrageenans (from seaweed), alginate (from brawn seaweed), pectin (from plant cell wall), agarose (from agar), and mixtures of xanthan (produced by fermentation of xanthomonas campestris on a glucose medium) and galactomannan (from locust bean gum).

The gelling mechanism for typical polysaccharides can be divided into three types according to the nature of the junction zones, ie. multiple helices, ribbon and micelle junctions. Junctions involve extensive segments from two or more polymer molecules and form ordered structures. Junctions are not point interactions, not whole chain interactions but segment interactions.

1. Multiple Helices

Multiple helices refer to a kind of association between chains where two or more chains associate in a certain helical direction. Polysaccharides that form junction zone by this mechanism are carrageenans, agarose and amylose. As an example, iota-carrageenan represents a polysaccharide which follows this gelation mechanism. Carrageenans are alternating copolymers of 1,3-linked β-D-galactose and 1,4-linked 3,6 anhydro-α-D-galactose, containing varying amounts of sulphate ester. These copolymers contribute to the helical junction formation. Interruptions or "kinks" occur at various points in the chain which terminate double helix formation. Kinks occur when the regular 3,6-anhydro

galactose residues are replaced by galactose. Double helix conformation was characterized in solid state by x-ray fiber diffraction (Dea, 1982) as a three fold right handed double helix. It was believed that the linkage pattern introduced a twist into the molecule, giving rise to the double helical structure. The forces responsible for the formation of helices are dominated by hydrogen bonds. Double helices interact by kink point association to form weak gel networks. On the other hand, double helices can aggregate to form a cohesive gel network in the presence of potassium ions (Rees, 1969).

2. Ribbon Junction

Typical polysaccharides following the ribbon junction gelling mechanism are alginate and pectin. Alginates are the major structural polysaccharide of brown algae of the Phaeophycea family. They have more complex structures than the carrageenans. This is based on a linear polyuronic backbone with three types of block structure. These are poly-β-D-mannuronic acid (M), poly-α-L-guluronic acid (G), and mixed (MG) blocked containing both uronic acids (Fig.1).

It was believed that alginate gels were formed by simple ionic bridging of two carboxyl groups on adjacent polymer chains with calcium ions. This happened in between guluronic acid chain segments. From circular dichroism and conformation studies done on isolated M and G segments, large transitions were observed for polyguluronic blocks and insignificant changes for the polymannuronate blocks. This "egg box" association is therefore considered involving cooperative binding of Ca^{2+} ions between aligned polyguluronate ribbons (Dea,1982). Unlike carrageenan gels, the alginate gels are not thermoreversible (Oakenfull, 1987).

Pectin is the partial methyl ester of 1,4 linked poly-(α-D-galacturonic acid) (Fig.2). This structure is, however, complicated by interruption in the chain of 1,2-linked

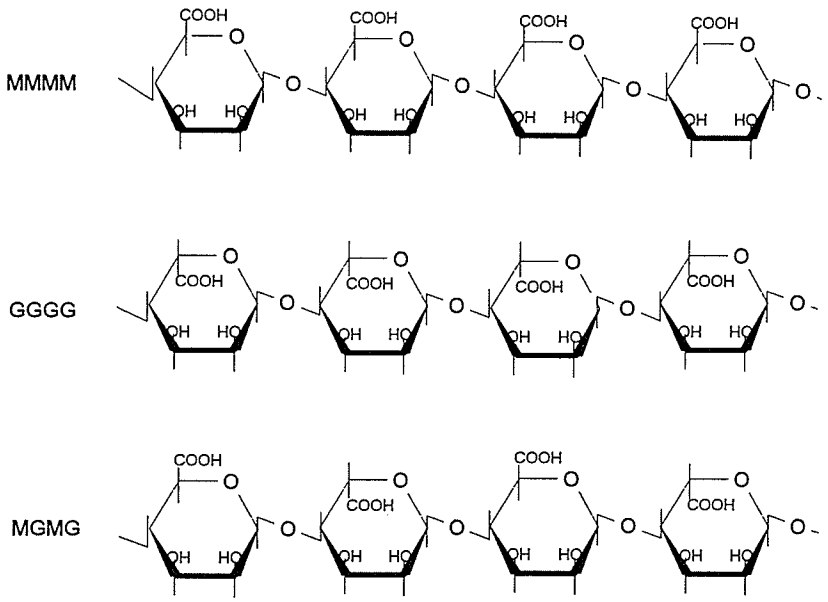
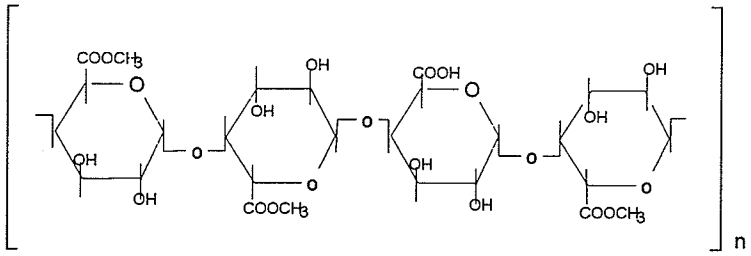


Figure 1. Structure of the polymer segments contained in alginic acid. M: β-D-mannuronic acid; G: α-L-guluronic acid.

A



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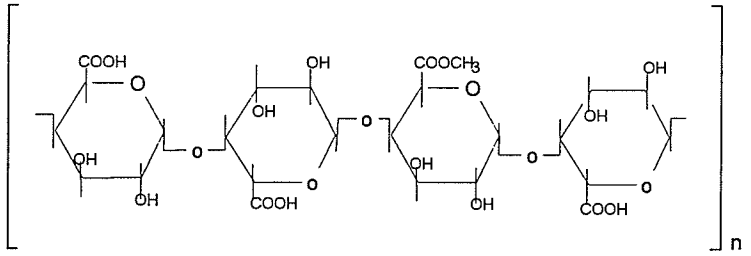

Figure 2. Chemical structure of pectins. A. High methoxyl pectin (degree of esterification 75%); B. Low methoxyl pectin (degree of esterification 25%).

α-L-rhamnose residues, and by neutral sugar side chains. Normally some of the carboxyl groups are esterified with methyl alcohol. A methoxyl content of about 14% is theoretically possible with 100% esterification. However, this is never achieved and most regular pectins have degrees of esterification of about 50-80% of theoretical (Glicksman, 1982).

Pectin can form two types of gels depending upon their degree of esterification (Glicksman, 1982). Regular, high methoxyl pectins will form gels at acid pHs and in the presence of a high concentration of sugar. Low methoxyl pectins are similar to alginate in gelling functionality, and require a divalent cation such as calcium in order to gel. These gels can be formed without sugar and over a wide pH range. This is not surprising since the polygalaturonate structure is almost the exact mirror image of polyguluronate except at the third carbon, and similar binding might therefore be expected.

3. Micelle Junction

Micelle junction refers to a gelling association between two different polysaccharides which usually cannot form gels as individuals (Dea, 1982). Examples of polysaccharide pairs which follow the micelle junction mechanism are mixtures of xanthan and galactomannan, and mixtures of agarose and galactomannan. Xanthan gum is a high molecular weight bacterial polysaccharide, produced by fermentation of Xanthomonas campestris on a glucose medium. The structure of xanthan consists of a cellulose backbone, substituted on alternate residues with trisaccharide side chains so that the repeating unit is a pentasaccharide. Like locust bean gum (source of galactomannan), xanthan gum alone will not form a gel. But together they will react to form a gel that has a very elastic, resilient texture with a very high rupture strength. The gels are thermally reversible, and show sharp melting and setting behaviour over a narrow temperature

range. Gel setting and melting points for xanthan and galactomannan gels have been found to increase with increasing total polysaccharide composition, but to show little dependence on the relative levels of the two polysaccharide (Dea,1982).

4. Role of Water in Network Formation

Polysaccharides are strongly hydrated in aqueous solution (Oakenfull, 1987). The polar chemical groupings that occur in polysaccharides (-OH, -O-, -COOH, $-COO^-$, OSO_3^-) obviously interact powerfully with the polar solvent. Some polysaccharides, such as methyl cellulose, also contain nonpolar groups introducing the possibility of hydrophobic effects. The single most important mode of interaction of carbohydrates with water is by hydrogen bonding from the sugar hydroxyl-groups (Oakenfull, 1987). The properties of the gel are the net result of the complex interactions between these two components. The water, as a solvent, influences the nature and magnitude of the intermolecular forces which maintain the integrity of the polymer network; the polymer network holds the water, preventing it from flowing away.

B. Chemistry and Gelling Mechanisms of Typical Globular Proteins

Globular proteins found in food systems include proteins from egg white (ovalbumin), soybean (7S, 11S globulins), milk (casein) and muscle (myosin). The ability of globular proteins to form gels and provide structural matrices for holding water, flavours, and sugar and food ingredients is useful in food applications and new product development. Gelation mechanisms of globular protein can be classified into two major types based on a two stage process and a corpuscular model.

1. Two Stage Process

The two stage process was first proposed by Ferry in 1948 (Ferry, 1948). In this process, protein molecules are induced to unfold and then refold so as to form a network. A force balance is important between repulsion and attraction forces of polypeptide chains (Arntfield et al.,1989). If the attractive forces between chains are low, denaturation during heating should be faster than chain association, and a fine gel network can result. An increase in attraction forces leads to gelation prior to sufficient accumulation of free chains, resulting in a coarse opaque gel. As attractive forces are further increased, only a precipitate is formed. Forces responsible for the gel structure are dominated by noncovalent forces such as hydrogen bonds, electrostatic interactions, Van der Waal's and hydrophobic interactions. On the other hand, covalent disulfide bonds also play an important role in gelation. The evidence of disulfide bond involvement can be seen from the formation of a 10% w/w ovalbumin/6M urea gel. The gel is resistant to urea, indicating existence of disulfide bonds (Ziegler and Foegeding, 1990).

Evidence to support this model comes from the Raman spectra for BSA which shows an increase in β sheet structure after gelation (Clark and Lee-Tuffell, 1986). Since β sheet is more extended than α helix, this could be an indication of unfolding. On the other hand, disulfide bonds provide linkages between polypeptide chains and could be evidence of refolding. Nevertheless, other noncovalent forces such as hydrogen bond, Van der Waals' forces and electrostatic and hydrophobic interactions could also be responsible for the network formation.

2. Corpuscular model

The corpuscular model was first introduced by Ma and Holme (1982). Protein molecules are induced to unfold and then soluble aggregates (limited aggregation) interact

to form a gel. In this case, large random aggregation results in shorter strands and coagulation rather than gelation. This model is different from the first model in the second stage in that instead of partly refolding, structures develop through limited aggregation. Using scanning electron microscopy to study glycinin from soy protein, the existence of a beaded or stranded gel structure has been noted (Beveridge et al., 1983). In addition, soluble aggregates have been observed prior to network formation (Nakamura et al.,1984). Furthermore, this model explains the high concentration required for protein gelation in comparison with gelling polysaccharides, since random aggregation requires higher concentrations (10-12 times) than chain interaction through junction zones (Poole and Fry, 1982).

3. Gelation of Canola Protein

Canola is an important cash crop in Canada. The oil is employed in the food industry while the commercial meal is used only as animal feed despite a well rounded amino acid composition. That canola protein is not used as food components is due to antinutritional factors including glucosinolates, phytic acid and phenolic compounds and to some poor aspects of functional properties. Canola protein has received great attention concerning its potential utilization as an edible component. Functional properties are an area in which researchers have focused on, besides the effort in eliminating the antinutritional properties. The ability of 12S canola protein to form gels was first demonstrated by Gill and Tung (1978). It was concluded that gelation occurred through a complex phenomenon involving both covalent and noncovalent forces. The gelation properties of canola protein were further characterized by several researchers. Work done by Léger and Arntfield (1993) revealed that hydrophobic forces and electrostatic interactions were responsible for the initial establishment of the canola gel network while

gel stabilization and strengthening were attributed to disulfide bonding, electrostatic interactions and hydrogen bonding. It was also concluded that gels prepared at alkaline pH were superior to gels prepared under acidic conditions. The effect of phytic acid and phenolic compounds on the protein functional properties of canola protein have been studied by Wong (1995) and Rubino (1995). In general, binding between these antinutritional factors and protein was found to be detrimental to the gelation properties of the mixture. The present research expanded research in the area of canola protein gelation into a multicomponent system.

4. Role of Water in Network Formation

The unique nature of water as a solvent is at its most obvious in the hydrophobic effects (Oakenfull, 1987; Chou and Morr, 1979). Nonpolar groups or molecules are surrounded by an ordered layer of water molecules. When these nonpolar molecules approach each other some of the ordered water molecules are squeezed out and the molecular rearrangements that this entails provide the thermodynamic driving force for hydrophobic interactions (Oakenfull, 1987). Similarly, in electrostatic interactions the hydrogen shells of ordered water molecules surrounding the ions are important. Association of ions involves interpenetration of these hydration shells with consequent rearrangement of water molecules (Oakenfull, 1987). Hydrogen bonding between solute molecules in water is also powerfully influenced by the solvent (Chou and Morr, 1979). Hydrogen bonding within and between biopolymers is therefore always a cooperative process with many individually weak interactions combining to maintain the integrity of a particular structure (Oakenfull, 1987). However, interactions between biopolymers and water were considered to be equal throughout the system and would be ignored when we looked at the interactions between biopolymers. This thesis focused on protein-

polysaccharide interactions and assumed water effect be the same throughout the system.

5. General Comparison of Gelling Mechanisms between the Polysaccharide and Globular Protein

Gelling mechanisms are important in understanding the relationship between bulk rheological properties of gels and the molecular properties of gel-forming polymers. While polysaccharide gelation usually follows a cold setting model, globular protein gelation usually follows a heat-setting model since denaturation causing unfolding of polypeptide chain by heating is normally a necessary step in the gelation process. The gelation mechanisms for the typical polysaccharide gels have been classified according to the nature of the junction zones, ie. multiple helices, ribbon, and micelle junctions. Polysaccharide gels are usually thermally reversible and characterised by their low polymer concentrations, fine texture and transparency due to the junction zones present. Such junction zones are probably absent in globular protein gels, which follow an aggregated dispersion model, either by a two stage mechanism composed of unfolding and partial refolding of the polypeptide chain, or by a corpuscular mechanism composed of unfolding and interaction of soluble aggregates to form a gel. Therefore a higher protein concentration is usually required to form a rigid gel structure (7-10%) compared to some of the gelling polysaccharides (1% or less) and the gels are heat irreversible (Arntfield, 1989). Forces responsible for the gelation of both systems are usually dominated by noncovalent bonds, such as hydrogen bonds, electrostatic, Van der Waals and hydrophobic interactions. In addition, covalent disulfide bonds also play a stabilizing role in the case of protein gels.

C. Protein Polysaccharide Interaction

1. Definition of Thermodynamic Incompatibility

Thermodynamic incompatibility has been described as a common phenomenon in protein-polysaccharide interactions based on the studies of over hundred of different protein polysaccharide systems (Tolstoguzov, 1989). Thermodynamic incompatibility was defined as a phenomenon where solution mixtures of biopolymers may undergo liquid-phase separation, with the macromolecular components concentrated mainly in different phases (Tolstoguzov, 1986; 1989). Justification of thermodynamic incompatibility was always based on a visual assessment of a liquid-phase separation, although separation techniques such as centrifugation and concentration determination for each phase were used in generating a quantitative description for the phase diagrams (Polyakov et al., 1980; Tolstoguzov et al., 1985). Therefore, thermodynamic incompatibility is obviously a relative concept defined by the kind of judgment used. A judgment by the naked eye could be different from a judgment made with the assistance of a microscope. This phenomenon is essentially opposite to the phenomenon of the formation of intermacromolecular complexes. It takes place under the conditions where the complex formation is inhibited. This means that mixtures of biopolymer solutions can undergo phase separation only under certain specific conditions, namely pH values, concentrations of biopolymers and ionic strengths which inhibit interactions between the different macromolecular species and encourage their self association, i.e. under the condition promoting interactions between macromolecules of the same type. Since the natures of biopolymers vary from type to type, the conditions under which incompatibility occur also vary. Therefore, it is important to specify the conditions when thermodynamic

compatibility is to be applied to certain mixtures. In this study compatible systems were generally applied to protein-anionic polysaccharide systems and incompatible systems were applied to protein-neutral polymer systems under defined concentration and pH values. It is important, however, to point out that compatibility will change if these conditions change.

2. Interaction and Gelation of Protein-Polysaccharide Mixture

The nature of protein polysaccharide interactions has been characterized by the thermodynamic compatibility (Tolstoguzov, 1986), binding behaviour through electrostatic attraction (Stainsby, 1980), and chemical interactions through covalent bonds such as disulfide bonds or the formation of anionic polysaccharide esters through amino groups in the polypeptide chain of the protein (Stainsby, 1980). The thermodynamic incompatibility of a mixed protein polysaccharide system was mainly judged by a phase separation with the macromolecules being concentrated in different phases (Tolstoguzov, 1986). Thermodynamic compatibility was dependent on thermodynamic parameters. Therefore, it is always achieved under defined conditions, such as macromolecular concentration, pH values and temperature of the system. Electrostatic interaction studies demonstrated that complexes could immediately form by mixing an anionic polysaccharide and a positively charged protein (Ledward, 1994). With two macromolecules bearing the same overall charge, it was also possible to form complexes under certain conditions (Imeson et al., 1977; 1978). The gelation properties of the mixed protein-polysaccharides have been conducted for many systems, including myofibrillar protein-polysaccharide (Xiong and Blanchard, 1993).

Using protein-polysaccharide interactions, processors can regulate gelation formation conditions along with rheological properties (Tolstoguzov, 1991). Titration-

complex of sodium caseinate and sodium alginate under conditions of low ionic strength at pH 4.5 will gel at complex concentrations over 7%. The gels are thermally stable at temperatures up to 80°C. Ionic strength, pH, time and composition all influence the final properties of the gel. For example, Tolstoguzov (1986) reported gelatin-alginate complexes melting at 30 to 40°C but after a 24 hour period the gels became thermally stable. With the addition of 0.3M NaCl and a pH greater than gelatin's IEP, the gels become thermally reversible with similar thermodynamic properties as gelatin (Tolstoguzov, 1986). Thus the physical dependence of the functional properties of the gel with time is due to the change in macromolecular interactions. Hughes and coworkers (1980) were studying the sodium alginate and sodium pectate gelation behaviour in the presence of meat proteins. Both polysaccharides are of similar structure, capable of forming gels with calcium ions and able to interact strongly with proteins. However, bovine serum albumin or myoglobin at 1% concentrations were able to interfere with the gelling mechanism for alginate but not for pectate. The effect increased with increasing pH. Because pectate has a higher affinity for calcium than does alginate, pectate competes more effectively with the protein for calcium and is able to provide a strong gelling network (Hughes et al., 1980). In the presence of sodium alginate, soy protein has been studied in term of emulsifying properties (Lippi and Taranto, 1981; Kiosseoglou and Doxastakis, 1988). Investigations on the protein polysaccharide interactions have led to several applications in food processing, such as protein recovery, purification and concentration (Imeson et al., 1978; Nakamura et al., 1992; Tolstoguzov, 1986; 1988; Samant et al., 1994). However, reports on the relationship between the interactions and the gelation properties were limited. Therefore, more work needs to be done in this area.

III. MATERIALS AND METHODS

A. Source of Materials

Bovine Serum Albumin (BSA), 96-99% albumin, was obtained from Sigma Chemical Co. (St. Louis, MO. USA).

Raw defatted canola meal was obtained from the CanAmera, Fort Saskatchewan, AB. The canola protein isolate was subsequently extracted from the meal using methodology involving the formation of a protein micellar mass (PMM) as outlined by Murray et al. (1978). 12S canola globulin was fractionated by gel filtration using canola protein isolate as a raw material. Protein content of canola protein isolate was 79%, while that of 12S canola globulin was 95% (Kjeldahl method). 12S canola globulin had a molecular weight of 300,000 daltons (Schwenke et al., 1987).

Pectin, Baker Grade, with a pH value of a 1% aqueous solution being 3.5, was obtained from J.T. Baker Chemical Co. Sodium alginate, with medium viscosity, was used in gel filtration. The viscosity of a 2% solution at 25 °C was 3500 centipoises (cps). Low viscosity sodium alginate was used in the turbidimetric analysis and the rheological test. The viscosity of a 2% solution at 25 °C was 250 cps. and the pH value of a 5% aqueous solution was 7.4. No effects of the viscosity of the alginates on the protein-polysaccharide interaction have been reported in the literature (Lippi and Taranto, 1981). Both sodium alginates were obtained from Sigma Chemical Co. Methyl cellulose, with a viscosity of a 2% aqueous solution at 25 °C being 400 cps. and the pH value of a 1% aqueous solution being 6.4, was obtained from Sigma Chemical Co. Guar gum, food grade, was obtained from Prescott Company, Mississauga, ONT. According to reference

(Whistler and Hymowitz, 1979), a 1% aqueous dispersion of guar gum has a viscosity of 3,000-6,000 cps.; osmotic pressure measurements on the triacetate indicate a molecular weight of 220,000 daltons. Xanthan was obtained from Kelco Corporate Headquarters, San Diego, CA. USA.

Unless stated otherwise, all chemicals used in this study were certified A.C.S. grade and supplied by Fisher Scientific Co. Nepean, ONT. Canada.

B. Canola Protein Extraction and Isolation

Canola protein was isolated from defatted canola meal using a modified version of protein micellar mass technique (Murray et al., 1978; Welsh, 1988; Burgess, 1991). Meal samples weighing 3500 g were stirred with 35 L of 0.5 M NaCl (1022.7 g) in a large kettle equipped with an overhead lighting mixer (model 10) at room temperature. After 4 h the kettle was rotated to pour the sample into 8 L plastic pails. This high salt protein extract (HSPE) was transferred into 1 L centrifuge bottles and centrifuged at 5°C using a Sorvall Refrigerated Centrifuge (model RC-3, Ivan Sorvall Inc. Norwalk. Conn., USA) at 3,000 g for 12 min. After centrifugation, the supernatant was vacuum filtered and placed in a large plastic pail and refrigerated at 4°C overnight prior to the concentration step simply because of the amount of time required for concentration. The HSPE was then concentrated with an Amicon Hollow Fiber cartridge (H10P30-20, Amicon Corporation, Danvers, MA, USA) with a 30,000 molecular weight cut-off. The Amicon ultrafiltration unit (UF) was operated with an inlet pressure less than 140 KPa (20 psi) and a backpressure of 50 KPa (0.5 bar). The volume of the HSPE was reduced approximately 16 fold. In the concentration mode, fluid was drawn from the reservoir and pumped via a pump to the hollow fibers and returned to the reservoir via the recirculating tubing. As the process proceeds, the low molecular weight species (water,

salt, glucosinolates, phenolics and phytates) passed through the fiber walls and emerge in the discarded permeate. The retained species were progressively concentrated in the reservoir. The HSPE was then diluted by 15 times its volume with cold city water. After sitting overnight in cold storage, the micelles formed were harvested by centrifuging at 5°C using a Sorvall Refrigerated centrifuge (model RC-3) at 3,000 g for 8 min. The supernatant was discarded and the pellet or PMM was placed in small containers with 0.2% ascorbic acid and frozen at -40 °C. Once frozen the samples were placed in a freeze-drier (model 10-146MP-BA S#1257, Virtis, Gardiner, NY. USA) for 1 week. The dried PMM samples were stored at 4°C until required for use.

C. Fractionation of 12S Canola Globulin

The 12S canola globulin and albumins are two major fractions in canola protein. The canola protein isolate PMM was used as raw material to further isolate the 12S canola globulin fraction from the albumins. For each batch, PMM sample weighing 0.1 g dissolved in 0.5M NaCl was applied to the K26/100 column packed with Sephacryl S-300 HR and eluted using 0.5 M NaCl. The fractions were collected and those containing the required protein pooled. The combined batches were dialyzed overnight against water and the purified 12S canola globulin was then freeze dried.

D. Sample Preparations

Three concentrations were examined for BSA (3, 5 and 10% w/w) and three for each polysaccharide i.e. sodium alginate, pectin and methyl cellulose (0.5 1.0 and 1.5% w/w). These levels were chosen based on preliminary experimentation. To ensure uniform sample preparation, each combination of protein and polysaccharide was prepared from an initial concentration of 20 or 10% (w/w) BSA and a 5% (w/w)

polysaccharide sol. Where necessary, pH values of the samples were adjusted using 0.5M HCl or 0.5M NaOH. Each mixture to be tested was allowed to stand overnight and tested on the following day to overcome any time effects. For gel filtration, pectin was adjusted to pH 6.0 before it was put on the column either alone or mixed with BSA. No pH adjustment was necessary for sodium alginate and methyl cellulose.

For gel filtration, samples were prepared separately from those used in rheological testing. Heat treatments were performed in a water bath at 70°C for 15 min for the combinations of BSA and polysaccharides to assess the effect of heat treatment on the binding behaviour. For canola protein isolate-polysaccharide systems, procedures were similar to the one for the BSA system except there were changes in protein concentration levels and pH values. The three levels of protein isolate concentrations were 5, 10 and 15% and all the experiments were conducted at pH 9. Samples were adjusted to the desired pH using 1.0 M NaOH before the rheological test. Higher concentrations for each level were used since protein content in canola protein isolate was lower (79%) than in BSA (95%) In addition to the polysaccharides used in BSA systems, guar gum was also used in canola protein isolate systems.

E. Turbidimetric Analysis

Turbidimetric analysis was conducted with a Hewlett Packard 8451 A Diode Array Spectrophotometer (Scientific Instruments Division, Palo Alto, CA., USA) at 520 nm to examine the formation of BSA-polysaccharide complexes. This wavelength was assessed by scanning both individual and the cloudy mixtures over the range of 250-650 nm. It was found that the maximum absorbance due to cloudiness occurred at this wavelength. This technique was used in previous works for rapeseed-phytic acid interaction also using 520nm (Schwenke et al. 1987) and tubulin self assembly

assessement using 350 nm (Andreu and Timasheff, 1986).

F. Gel Filtration

A K26/100 column packed with Sephacryl S-300 HR (Pharmacia Biotech AB, Uppsala, Sweden) in conjunction with a LKB 2212-010 HeliRac collector (LKB-Produkter AB, Bromma, Sweden) were used for gel filtration. A 0.1 M sodium phosphate buffer at a flow rate of 30 mL/h was used as eluent. For each sample, a five mL aliquot of protein, polysaccharide or their mixture was applied to the column. The eluted fractions were collected and analyzed for protein by determination of absorbance at 280 nm and for polysaccharide by determination of absorbance at 480 nm (sodium alginate and pectin) or 490 nm (methyl cellulose) following a phenol-sulphuric reaction (Southgate, 1991). Results were reported as absorbance against fraction number in elution profile diagrams. Each fraction collected had a volume of about 8 mL.

G. Visual Assessment

All the samples for gel filtration were noted as clear, translucent or cloudy before and after heat treatment. All the samples for rheological testing were examined for phase separation before testing, and gels after testing were classified as translucent or opaque. Visual assessment provided the justification of thermodynamic compatibility. The justification was done at room temperature on samples hold in 10 mL test tube. According to the definition that thermodynamic incompatibility is a phenomenon where the solution mixtures of biopolymers undergo liquid-phase separation, with the macromolecular components concentrated mainly in different phases (Tolstoguzov, 1986; 1989), systems would be considered incompatible if a liquid two phase separation was noted.

H. Rheology

A Bohlin VOR rheometer (Bohlin Reologi, Inc., Edison, NJ), operated in the small-amplitude oscillatory mode, was used to follow protein gel formation during heating and cooling and to characterize the rheological properties of the resulting gels (Arntfield et al., 1990a; 1990b).

The rheometer was equipped with 30-mm parallel plate geometry and with a torque bar calibrated to 93.1 gcm. Input strain amplitude for dynamic analysis was 0.02. All the samples of mixed protein-polysaccharide were prepared the day before and allowed to stand overnight at room temperature before conducting the rheological test. Approximately 1 mL of sample was applied to the lower platen of the parallel-plate geometry so that a gap of 1.00 mm was realized when the upper platen was lowered. A 16-cm strip of masking tape was wrapped around the circumference of the cylinder supporting the lower platen to form a well. Mineral oil was added to the constructed well until the upper platen was covered to prevent sample drying during heating. Once in place, samples were subjected successively to a heating phase, a cooling phase and a frequency sweep.

Samples were heated and then cooled over a temperature range of 25-95 °C at a rate of 2°C/min, followed by a frequency sweep at 25°C. A frequency of 0.10 Hz was used for the thermal scans. Rheological data were collected every 2.0 min with a thermal equilibrium time of 10s. At the end of each phase, the final temperature was held for 2.0 min. Frequency sweeps of the final product were conducted over a range of 0.01-10 Hz at 25°C. The G' and tan δ values at 1 Hz of the final frequency sweep were taken as the rheological results of the gelling products, and reported in tables of the text. Rheological properties were expressed in terms of storage modulus (G') and loss modulus (G").

loss tangent was calculated manually as tan δ = G"/G'.

The storage modulus (G') is a measure of the elastic component of the network and represents the type of structure contributing to a three dimensional network. The loss modulus (G"), on the other hand is a measure of viscous component and may represent interactions which do not contribute to the three dimension nature of the network. As a result, a change in tan δ should be indicative of the type of network formed in that lower tan δ values represent better three dimensional structures (Arntfield et al., 1990a)

I. Statistical Analysis

All data were reported as a mean of duplicates. A randomized complete block design (3x3) was used for the rheological test. The analysis of variance by SAS system release 6.09 in conjunction with Duncan's multiple range test were conducted to see if there was a significant difference among samples in the test. Results of (3x3) randomized complete block design were shown in overall evaluation and expressed as the effect of protein and the effect of polysaccharide on storage modulus G' and tan δ. Control samples were not included in the randomized complete block design. Row data were then compared with the control individually within each protein concentration level and also done the SAS within each polysaccharide concentration level. These results were reported as single effects in either row or column in tables of the text. All comparisons were made among means. The standard error represented standard error of means. Only ANOVA data for the (3x3) randomized complete block design for each protein-polysaccharide system were listed in Appendices. ANOVA for each individual level of concentrations were done for the comparison but the raw data were not given.

IV. RESULTS

A. BSA-Polysaccharide Systems

1. Turbidimetric Analysis

Complexes formed upon mixing protein and anionic polysaccharides at pH values below the IEP of BSA (4.5). This phenomenon was accompanied by cloudiness and further precipitation of the complexes. As shown in Fig.3, the change in turbidity of the mixture is sharp across the protein's IEP. The pH value around the IEP is critical in determining the complex formation between the biopolymers. Proteins carry a net positive charge at pH values below the IEP, and this charge is responsible for the interaction with the anionic polysaccharides, which carry the opposite negative charge. Previous research in this area was done on protein-dextransulphate mixtures using nephelometric analysis based on light scattering under 90° (Gurov et al., 1977, 1978) and similar results were obtained.

A comparison of sodium alginate and pectin shows similar patterns, suggesting a common phenomenon involving electrostatic attractions between BSA and anionic polysaccharides. However a slight shift towards a lower pH and a weaker response was observed for pectin, indicating pectin may have a weaker interaction than does sodium alginate. This is probably accounted for by less accessible anionic groups in pectin due to methylation of carboxyl groups which limits the amount of free carboxyl radicals. In contrast, sodium alginate has more free carboxyl radicals available, based on the structures of pectin and alginate reported (Dea, 1982) and stronger interaction can be

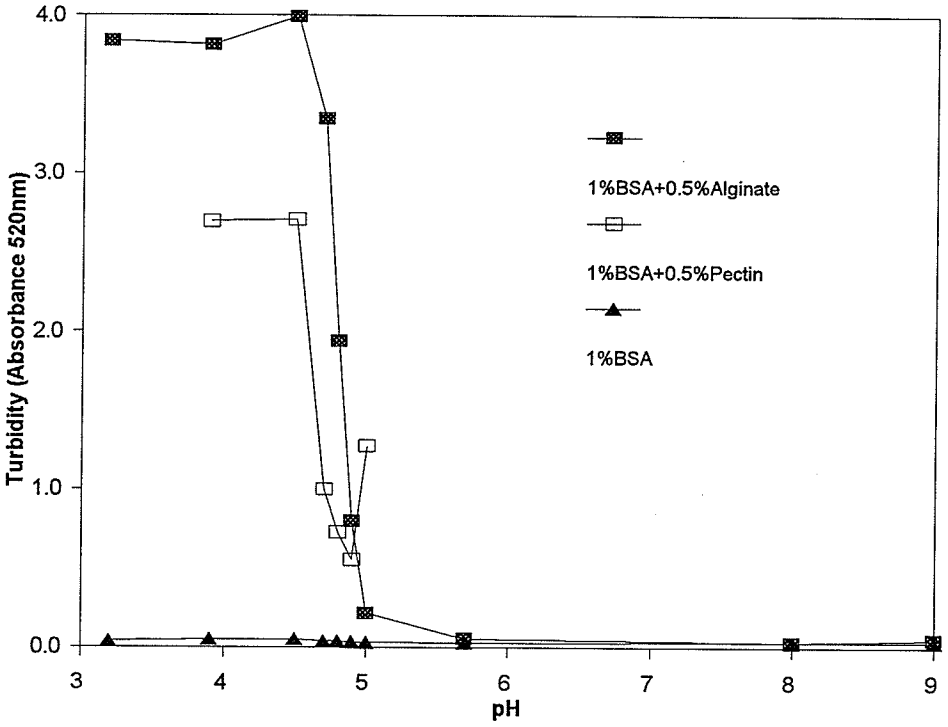

Figure 3. Turbidity of BSA-anionic polysaccharide mixtures in relation to pH values.

expected.

In manipulating the pH value of the system, it must be noted that the pectin used in this study has a low natural pH value (1% w/w pectin water solution, pH=3.5). Therefore, the mixing of pectin-protein systems always resulted in the formation of insoluble complexes at their natural pH. On the other hand, sodium alginate exhibits an intermediate pH (5% w/w sodium alginate water solution, pH=7.4), so that insoluble complexes will not form without adjusting the pH value. The presence of insoluble complexes can have negative impacts on both elasticity (G') and network (tan δ) properties, as will be shown in the result of gelation properties at pH below protein isoelectric point, though in some systems (sodium alginate-soya isolate) similar mechanisms contributed an improvement in emulsification properties (Lippi and Taranto, 1981). A similar phenomenon was that mixture complex had poorer gelation properties than titration complex (Tolstoguzov, 1986).

2. Gel Filtration

All combinations of BSA-polysaccharides were run on a gel filtration column using a 0.1 M sodium phosphate buffer at pH 6.0. Elution profiles of BSA-sodium alginate, -pectin, and -methyl cellulose are shown in Figs.4 to 9.

a. BSA-Sodium Alginate and -Pectin Systems

Binding was observed with heated conditions (70 °C,15min) but not with unheated conditions for anionic polysaccharides at pH 6.0 (Figs. 4 to 7). Under heated conditions, the elution profile for the BSA shifted towards a lower volume fraction in the presence of both alginate and pectin (Figs. 5,7). Furthermore, elution profiles for BSA and the polysaccharide were equivalent in their response, indicating a molecular binding exists

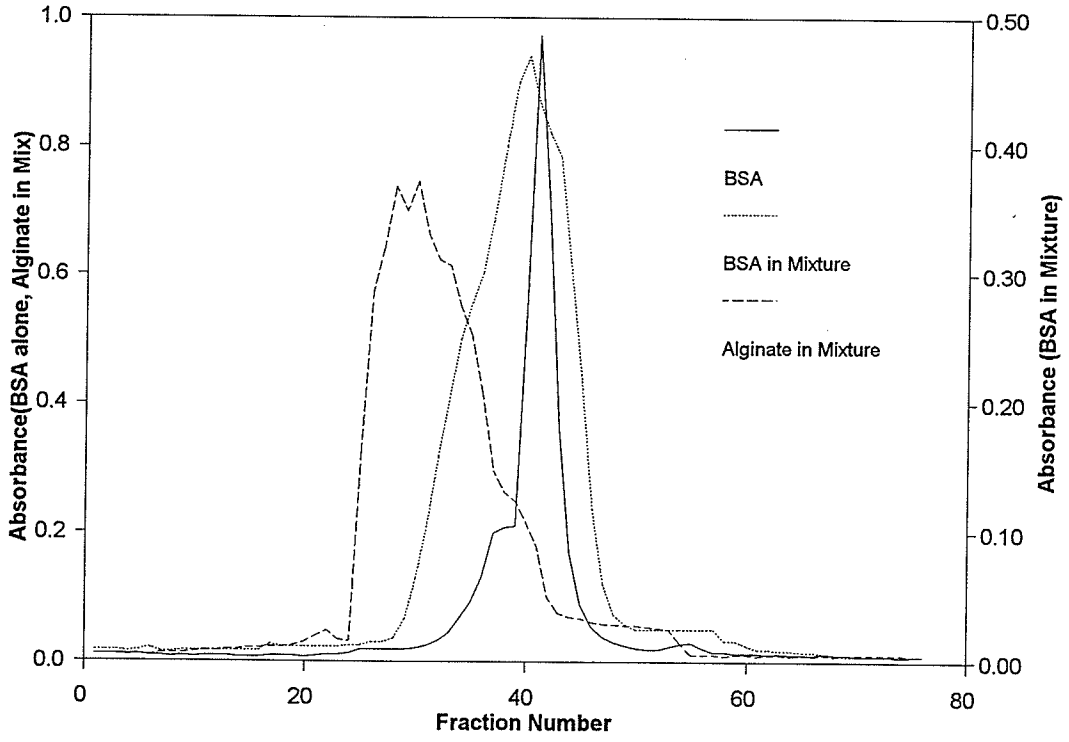

Figure 4. Elution profile of BSA alone and BSA-sodium alginate mixture unheated. Sample size: 5.0 mL of 1.0% BSA alone, 5.0 mL mixture of 1.0% BSA and 1.0% sodium alginate. Each fraction had a volume of 8.0 mL. Eluent: 0.1 M phosphate buffer, pH 6.0.

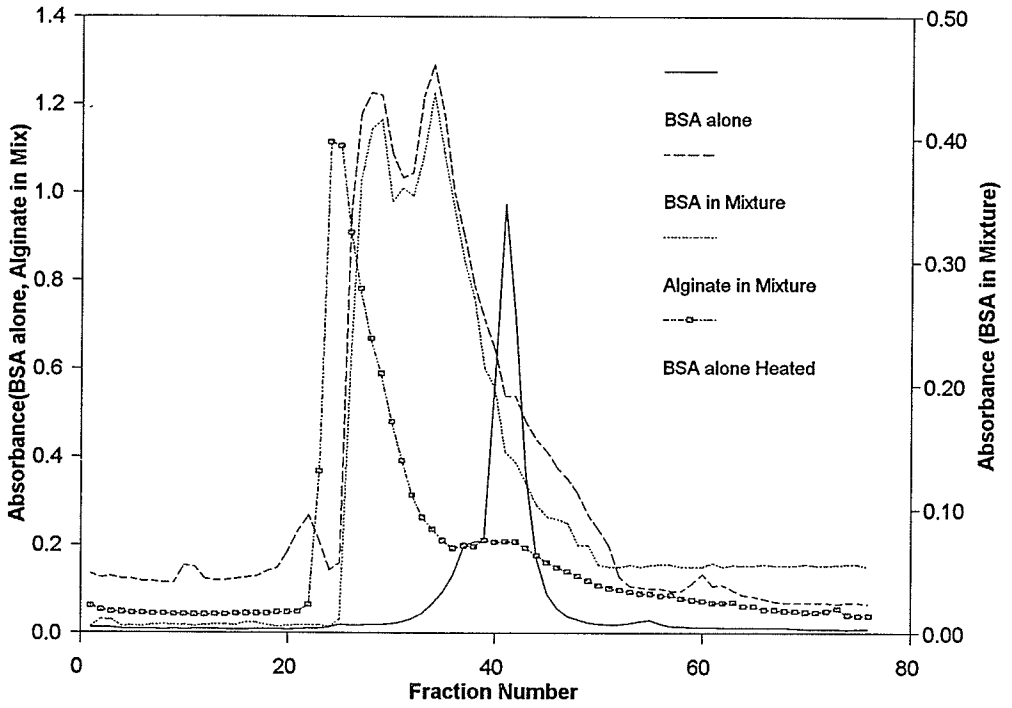

Figure 5. Elution profile of BSA alone and BSA-sodium alginate mixture after heat treatment. Sample size: 5.0 mL of 1.0% BSA alone, 5.0 mL mixture of 1.0% BSA and 1.0% sodium alginate heated at 70°C for 15min. Each fraction had a volume of 8.0 mL. Eluent: 0.1 M phosphate buffer, pH 6.0.

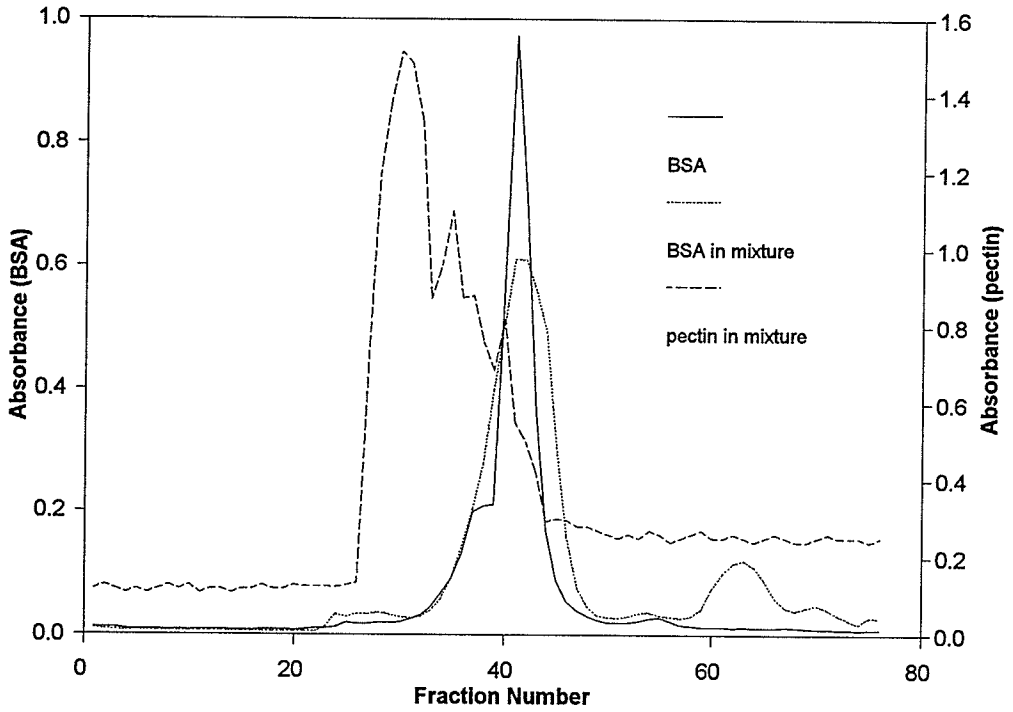

Figure 6. Elution profile of BSA alone and BSA-pectin mixture unheated. Sample size: 5.0 mL of 1.0% BSA alone, 5.0 mL mixture of 1.0% BSA and 1.0% pectin. Each fraction had a volume of 8.0 mL. Eluent: 0.1 M phosphate buffer, pH 6.0.

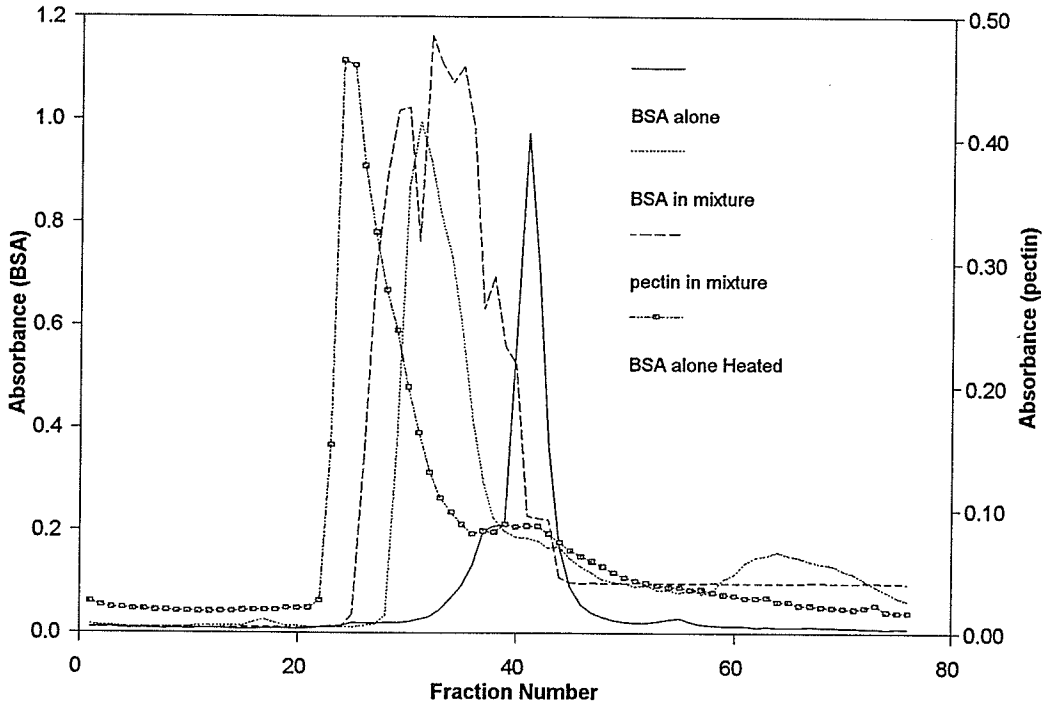

Figure 7. Elution profile of BSA alone and BSA-pectin mixture after heat treatment. Sample size: 5.0 mL of 1.0% BSA alone, 5.0 mL mixture of 1.0% BSA and 1.0% pectin heated at 70°C for 15min. Each fraction had a volume of 8.0 mL. Eluent: 0.1 M phosphate buffer, pH 6.0.

between the biopolymers. Under unheated conditions (Figs 4,6), however, peaks of BSA and polysaccharide were well separated, indicating there was no binding. BSA peaks were broader in a mixture than in pure BSA solution probably due to an increase in viscosity in the presence of the polysaccharide. Although the concentrations of 1% BSA and 1% polysaccharide in this study were higher than in a previous study (Imeson et al., 1977), results from both studies were similar.

A change in appearance of the mixture was noted from its natural colour to a grey gel-like translucent appearance for BSA-sodium alginate and white cloudiness for BSA-pectin and BSA-methyl cellulose. These provide evidence of possible different mechanisms of interaction for alginate and pectin systems.

At least two mechanisms for binding of mixed alginate gel have been put forward. These are electrostatic attraction and covalent binding (Lippi and Taranto, 1981). Besides the electrostatic attraction which is common to all type of anionic polysaccharides and results from the interaction of a protein with a net positive charge and a negatively charged polysaccharide, there is another type of electrostatic attraction that happens where micro-regions of positive charges exist in the peptide chain of a protein that has an overall net negative charge (Lippi and Taranto, 1981; Imeson et al., 1977). The first type of interaction occurs with a protein system at a pH below the IEP, whereas the second applies to pH values above IEP but where the protein chain has been unfolded by some kind of force, such as heat. Micro-regions of positive charges exposed upon heating can provide sufficient interaction space to accommodate polysaccharide molecules. Covalent bonding, on the other hand has been reported to be favoured at $pH > 9.5$ for the ester alginate system (Stainsby, 1980). An indication of covalent bonding of a biopolymer is its thermo-stability. During heat treatment at a heating rate of 2 °C/min, BSA-sodium alginate did not precipitate, as was the case of BSA-pectin. This may indicate a more

thermostable product. While electrostatic attraction through micro-regions of positive charges should be responsible for the pectin system, it is reasonable to expect covalent binding to be a factor for the alginate system. However, more detailed work must been done before pathways can be realized.

b. BSA-Methyl Cellulose Systems

In the BSA-methyl cellulose system, no binding was observed under either the unheated or heated conditions, as shown in Figs. 8 and 9. Absorbance of BSA did shift towards a lower fraction volume under heated conditions. However, this was due to the denaturation and aggregation of the protein itself, as confirmed by a similar shift of heated BSA alone and the nonequivalent response between BSA and methyl cellulose in the heated system. There was no evidence of interaction. The results were in contrast with some reports where an interaction was observed following dry heat treatment for neutral polysaccharide systems, such as BSA-dextran (Harding et al., 1992) and ovalbumin-dextran (Kato et al., 1990). The incompatibility of BSA-methyl cellulose was not illustrated by gel filtration alone but further demonstrated by the visual assessment in that a phase separation was observed at a concentration of BSA above 3% and methyl cellulose above 0.5%. According to visual assessment, the incompatibility increased with the increase in biopolymer concentrations.

The importance of these elution profiles for the heated mixtures lies in the fact that gelation occuring during rheological testing involves a heating phase and, therefore, the molecular binding behaviour seen from these diagrams should also occur during gelation.

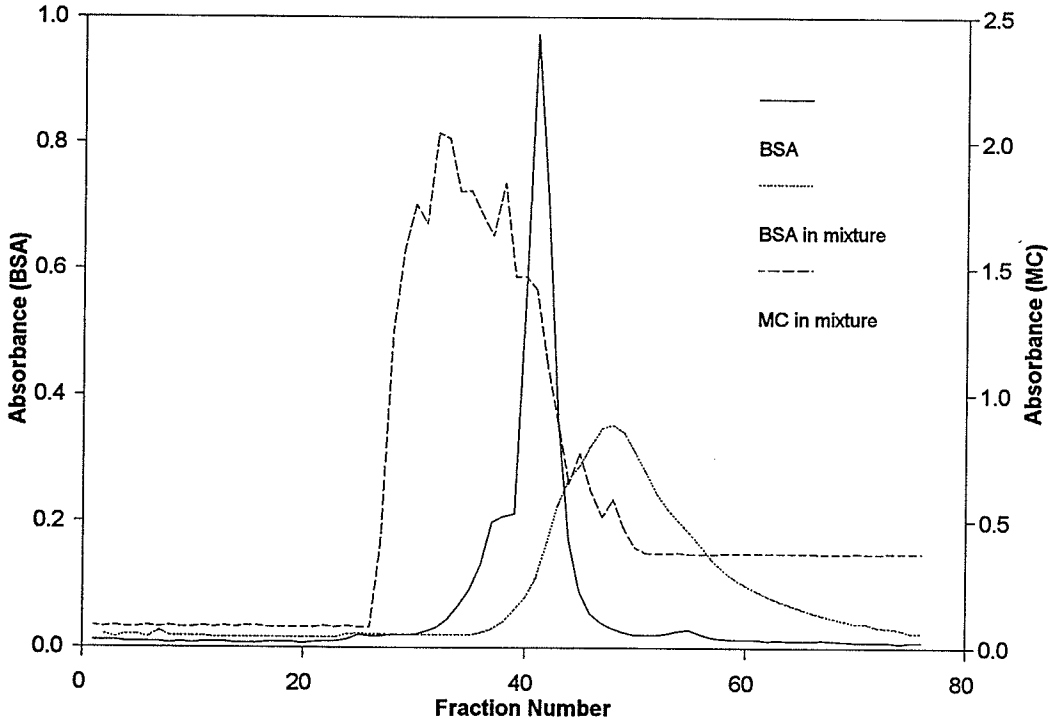

Figure 8. Elution profile of BSA alone and BSA-methyl cellulose (MC) mixture unheated. Sample size: 5.0 mL of 1.0% BSA alone, 5.0 mL mixture of 1% BSA and 1.0% methyl cellulose, each fraction had a volume of 8.0 mL. Eluent: 0.1 M phosphate buffer, pH 6.0.

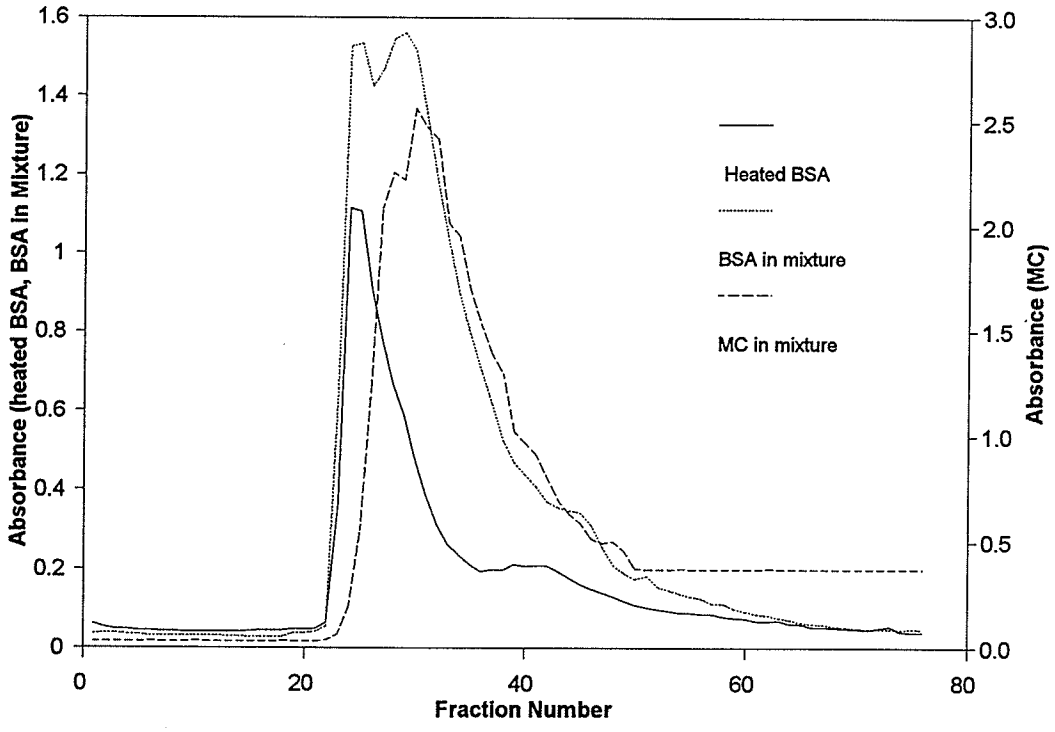

Figure 9. Elution profile of BSA alone and BSA-methyl cellulose (MC) mixture after heat treatment. Sample size: 5.0 mL of 1.0% BSA alone heated at 70°C for 15min, 5.0 mL mixture of 1.0% BSA and 1.0% methyl cellulose heated at 70°C for 15min. Each fraction had a volume of 8.0 mL. Eluent: 0.1M phosphate buffer, pH 6.0.

3. Visual Assessment

Table 1. Results of visual assessment for BSA-polysaccharide systems at different conditions.

Systems	Conditions				
	pH 4.7, for turbidity	pH 6.0, for gel filtration		pH 5.4-5.7, for rheology	
		unheated	heated 70°C,15 min	before test	resulting gel
BSA-Alginate	cloudy	clear	translucent liquid	viscous liquid	translucent gel
BSA-Pectin	cloudy	clear	cloudy	viscous liquid	white opaque gel
BSA-Methyl Cellulose	-----	clear	cloudy	2 phase	slurry or opaque gel

Results of the visual assessment are given in Table 1. Samples for turbidimetric analysis and gel filtration had a concentration of 1% for BSA and 0.5-1% for polysaccharide. A common precipitation phenomenon was observed for anionic polysaccharide at pH values below the IEP of BSA (pH 4.9). At pH values above the IEP and when samples were heated, as was the case with gel filtration, a translucent liquid was observed for sodium alginate system and white cloudiness for pectin and methyl cellulose systems.

In the case of samples for rheological tests, the BSA concentration ranged from 3-10% and polysaccharide from 0.5-1.5%, resulting in a viscous material upon mixing. A phase separation was always observed for the BSA-methyl cellulose system, especially at high concentrations of the biopolymers. After the mixtures were heated and cooled,

translucent gels were always observed for alginate systems, regardless of the concentration of BSA. White opaque gels were observed for BSA-pectin at this pH value. It must be noted, however, that at pH values below the IEP, a white slurry resulted, except at a high concentration of BSA; in this case a white opaque gel resulted. With the BSA-methyl cellulose system, a slurry like material was obtained at low BSA concentrations while a white opaque gel resulted at high protein concentrations.

Combining visual assessment with the turbidimetric and gel filtration data results in the conclusions that BSA-anionic polysaccharides form a compatible system either through electrostatic force or possible through covalent bonding, whereas a BSA-methyl cellulose system forms an incompatible system, which causes a phase separation and the nonbinding behaviour observed in gel filtration.

4. Rheology

a. Sodium alginate systems

The rheological data of mixed BSA-sodium alginate systems are given in Tables 2a-b. Data were based on a frequency of 1 Hz and at a natural pH value (around pH 5.7). The results of the analysis of variance showed that BSA concentration, sodium alginate concentration (Table 2a) and an interaction (Appendix Table A1) between these parameters all had significant effects on the G' values for the mixed gels ($p<0.05$). Increasing the concentration of BSA or sodium alginate in the tested concentration range both resulted in significant increases in G' values. The significant interaction effect indicated that increases in G' as a result of increasing BSA concentration were dependent on the concentration of sodium alginate. In comparison with the control BSA, however, a slight decrease was observed for low concentrations of these combination, specifically

Tables 2a-b. Rheological results of (a) storage modulus G' (Pa) and (b) tan δ for mixed BSA-sodium alginate gel at 1Hz*

(a)

BSA (%)	Sodium Alginate (%)				
	control	0.5	1.0	1.5	SE
3	420^{c1}	90^{a1}	213^{ab1}	295^{bc1}	40
5	652^{b1}	231^{a1}	1080^{c2}	1810^{d2}	93
10	1485^{a2}	6895^{b2}	19900^{c3}	25300^{d3}	503
SE	164	535	148	124	

(b)

BSA (%)	Sodium Alginate (%)				
	control	0.5	1.0	1.5	SE
3	0.132^{a1}	0.233^{b1}	0.176^{ab1}	0.250^{b1}	0.021
5	0.146^{a1}	0.173^{a1}	0.140^{ab12}	0.101^{b2}	0.010
10	0.137^{a1}	0.098^{b2}	0.093^{b2}	0.089^{b2}	0.004
SE	0.003	0.016	0.016	0.015	

* Column values with the same numbers are not significantly different, row values with the same letters are not significantly different ($p < 0.05$). SE = Standard error of means.

3% BSA and all levels of sodium alginate and the 5% BSA/0.5% sodium alginate combination. In contrast, a significant increase in G' was always observed for high

concentrations (10%BSA, 5%BSA/1.0, 1.5% sodium alginate) of each combination. In terms of gelation pattern, typical gelation diagrams are shown in Fig.10 for BSA alone at various concentrations and in Fig.11 for the mixtures of various concentrations of BSA and 1% sodium alginate. In the heating phase, the control BSA samples exhibited a similar pattern of structure development starting at around 70 °C. As seen in Fig. 10, the increases in G' values after this temperature were directly proportional to their protein concentration. During the initial cooling stage, G' values levelled off and then started to increase dramatically at temperatures around 50-60 °C. The extent of this increase was again dependent on concentration. This increase reflects the strengthening of H-bonding in the mixture, since lowering temperature favoured H-bonding (Léger and Arntfield, 1992).

Structure development for combinations of BSA-sodium alginate started much later, around 80 °C or above. It is interesting to note that the combination of the lowest concentrations of BSA and sodium alginate resulted in particularly late structure development at around 90 °C. This information suggests that the molecular binding enhances the macromolecular stability and may prevent macromolecules from associating. The total concentration of the biopolymers was critical in determining this behaviour.

In addition to the increased interaction that was noted with the pure protein system, the presence of sodium alginate seemed to have a synergistic effect. For the sample containing 10% BSA, the G' value at the conclusion of the heating phase was approximately 20 times higher when 1.5% sodium alginate was present. A different cooling phase was also observed for mixed BSA-sodium alginate, in comparison with the pure BSA. The period of levelling off in initial cooling stage was compressed. Instead, there was a steady increase in G' after a small plateau. This behaviour was most evident at higher BSA concentrations. With the 3% BSA concentration, however, the curve

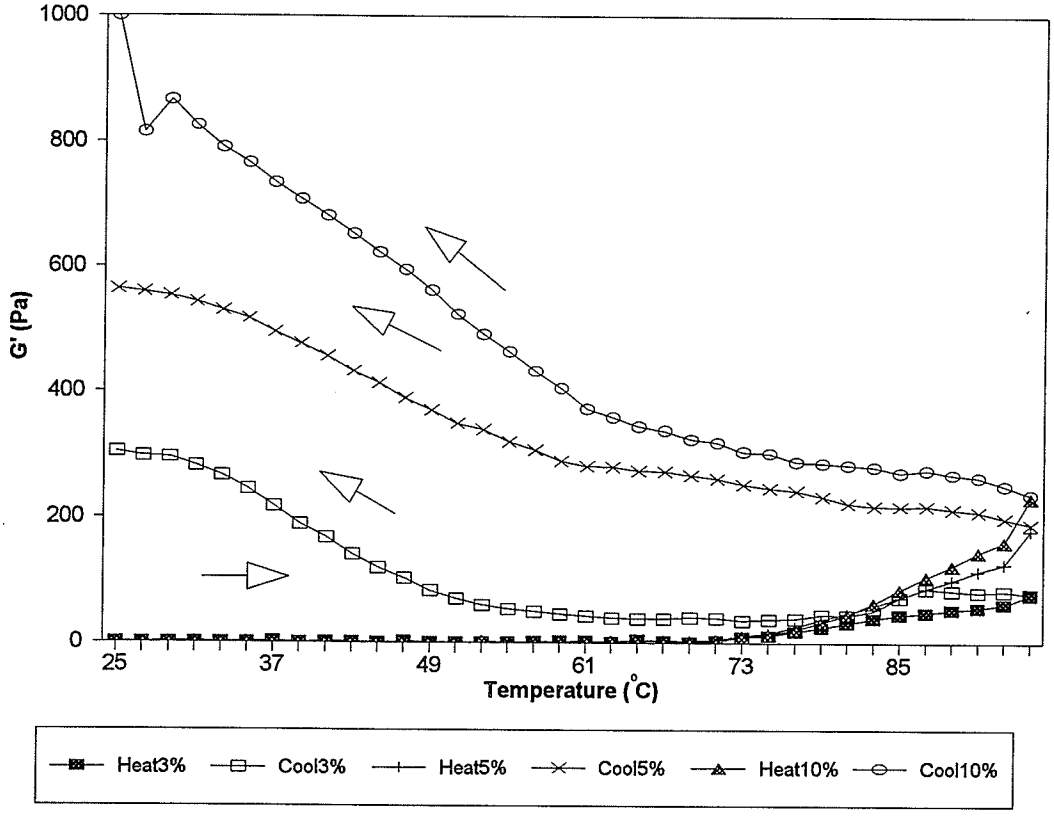

Figure 10. Storage modulus G' of BSA in relation to different BSA concentrations under heating and cooling phases of the rheological tests.

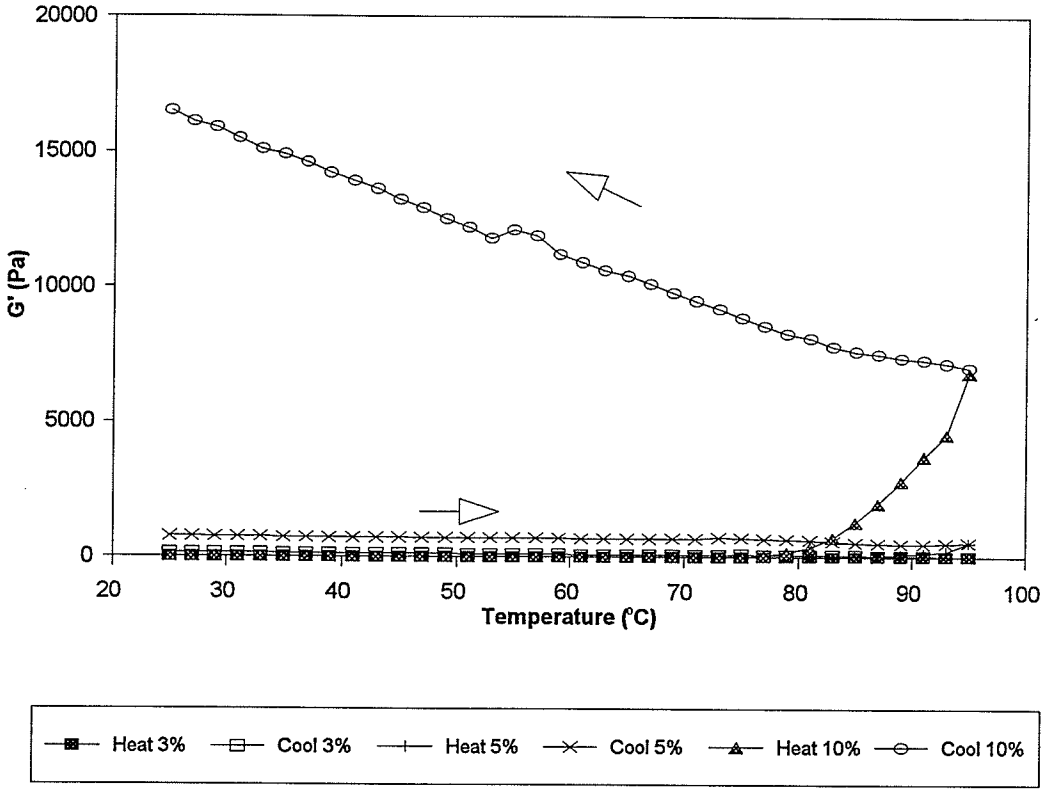

Figure 11. Storage modulus G' of BSA in the presence of 1.0% sodium alginate in relation to different BSA concentrations under heating and cooling phases of the rheological tests.

appeared to plateau after temperatures below 70 °C. It is possible that the difference in cooling pattern between control BSA and mixed BSA-sodium alginate results from the increasing role of hydrophobic interactions in gel formation contributed by sodium alginate (Tolstoguzov, 1991).

In terms of tan δ, a parameter which reflects the relative elasticity of the gel, BSA and sodium alginate concentrations were found to have significant effects (Table 2b). High concentrations of BSA in the mixture resulted in a lower tan δ, meaning a better gel structure (Arntfield et al., 1990a). Sodium alginate also had a significant effect on tan δ. Tan δ appeared to decrease with increasing sodium alginate concentration (Table 2b). It should be noted that in the control BSA samples, concentration does not have a significant effect on tan δ.

b. Pectin Systems

Rheological data for mixed BSA-pectin gels are shown in Table 3a-b at a natural pH (around pH 4.7) and at a frequency of 1 Hz. The analysis of variance showed that BSA concentration, pectin concentration and the interaction (Appendix Table A2) between them all have significant effects on G'. With 5% BSA, the samples containing pectin all had lower G' values, though for some unknown reason the lowest value was obtained with 0.5% pectin. This general negative effect may be attributed to the formation of more insoluble complexes at higher pectin concentrations. The insoluble complexes result from the electrostatic attraction at pH values below the IEP. It would appear that these complexes do not exhibit good gel properties, although other properties could have been improved in this way, such as increased emulsification properties as have been reported in previous work for soy isolate-sodium alginate mixture (Lippi and Taranto, 1981). Increased BSA concentration resulted in increased G' values for all

Tables 3a-b. Rheological results of (a) storage modulus G' (Pa) and (b) tan δ for mixed BSA-pectin gel at 1Hz.*

(a)

BSA (%)	Pectin (%)				
	control	0.5	1.0	1.5	SE
3	420^{b1}	11^{a1}	9^{a1}	3^{a1}	26
5	652^{b1}	33^{a1}	186^{a1}	95^{a1}	47
10	1485^{b2}	981^{b2}	166^{a1}	1590^{b2}	176
SE	164	109	54	58	

(b)

BSA (%)	Pectin (%)				
	control	0.5	1.0	1.5	SE
3	0.132^{a1}	0.121^{a1}	0.412^{a1}	0.520^{a1}	0.165
5	0.146^{a1}	0.171^{ab1}	0.170^{ab1}	0.177^{b1}	0.007
10	0.137^{a1}	0.179^{a1}	0.092^{a1}	0.155^{a1}	0.038
SE	0.003	0.019	0.140	0.135	

* Column values with the same numbers are not significantly different, row values with the same letters are not significantly different ($p<0.05$). SE = Standard error of means.

samples, although the increases in the presence of pectin was not as great as for the pure BSA.

Tan δ values for BSA-pectin gels were generally unaffected by BSA or pectin concentration, as shown in Table 3b.

It must be pointed out that increasing the pH values above the protein IEP for BSA-pectin system resulted in a completely different gelation pattern, which was believed to be similar to the one obtained with sodium alginate though the magnitude of the effect was not as great. Fig. 12 shows the effect of pH values on both sides of the IEP on G' and tan δ for both sodium alginate and pectin systems. At a pH value above the IEP, both anionic polysaccharides resulted in increases in G' and decreases in tan δ, indicative of good gel networks. At pH values below the IEP the results were opposite.

c. Methyl Cellulose Systems

Tables 4a-b show the rheological properties of mixed BSA-methyl cellulose. According to the statistical analysis, BSA and methyl cellulose concentrations have significant effects on the G' values and tan δ values. At low BSA concentration (3 and 5%), G' values decreased while tan δ increased in the presence of methyl cellulose. At high BSA concentration (10%), however, G' values significantly increased while tan δ remained unchanged in the presence of 1.5% methyl cellulose. At low BSA concentrations, the mixture exhibited gelation properties typical of methyl cellulose. A gel formed upon heating due to hydrophobic interaction contributed by methyl groups in the cellulose macromolecules. Upon cooling, however, the mixture exhibited again a viscous fluid property, as shown in Fig.13 and 14. The curve for methyl cellulose in the mixture (Fig.13) resembled the pure methyl cellulose curve shown in Fig.14. At high concentrations, however, the mixture exhibited gelation properties produced by the protein, as shown in Fig.15. Nevertheless, at sufficiently high BSA concentration methyl cellulose contributes in some way a synergistic effect to the gel network and causes an

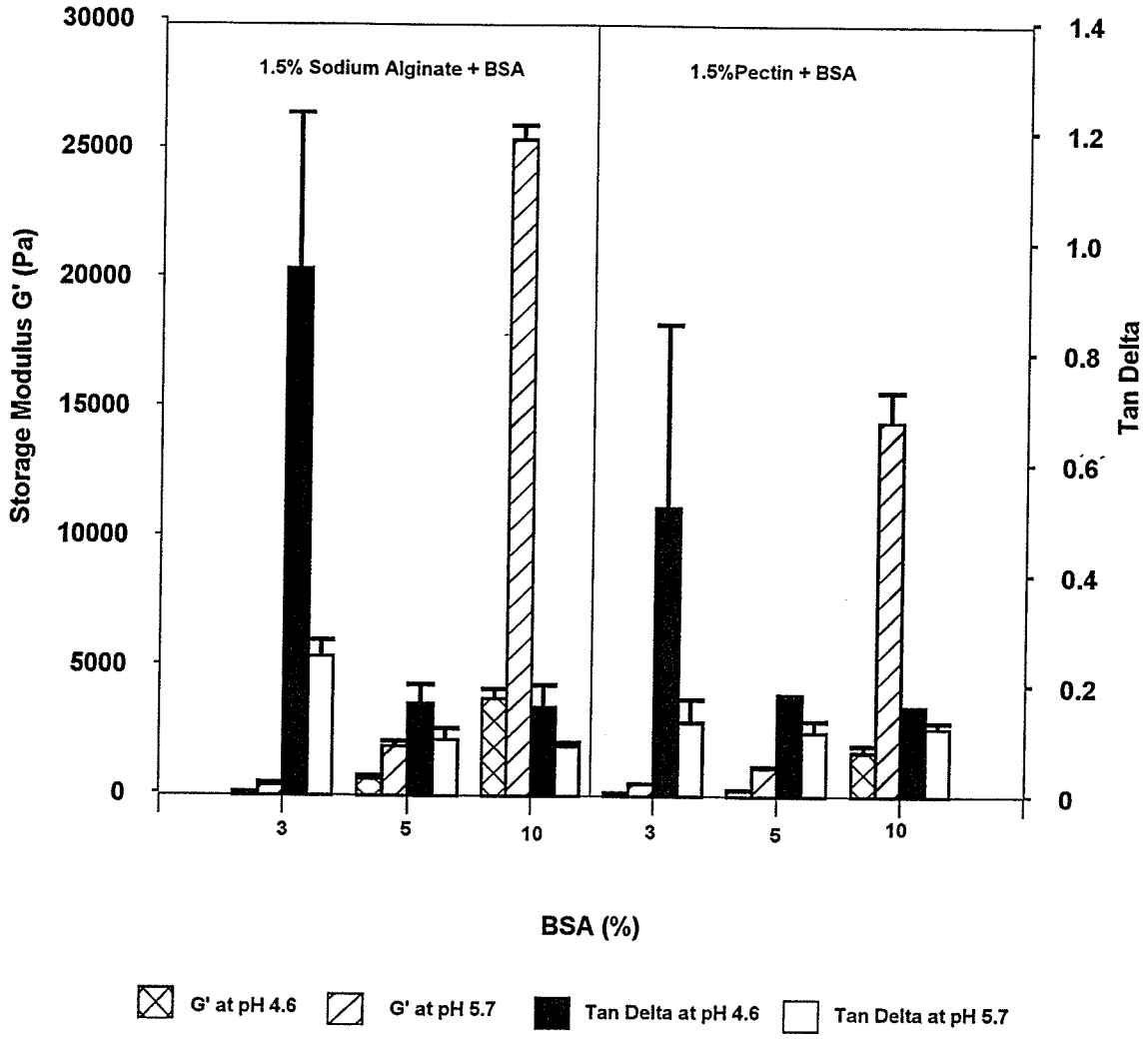

Figure 12. Effect of pH at below and above BSA isoelectric point (pH 4.9) on the storage modulus G' and tan δ of BSA-anionic polysaccharide systems at 1Hz.

Tables 4a-b. Rheological results of (a) storage modulus G' (Pa) and (b) tan δ for mixed BSA-methyl cellulose gel at 1Hz*

(a)

BSA (%)	Methyl Cellulose (%)				
	control	0.5	1.0	1.5	SE
3	420^{b1}	86^{a1}	26^{a1}	12^{a1}	48
5	652^{b1}	23^{a1}	14^{a1}	21^{a1}	26
10	1485^{a2}	1110^{a2}	1410^{a2}	4295^{b2}	174
SE	164	119	13	55	

(b)

BSA (%)	Methyl Cellulose (%)				
	control	0.5	1.0	1.5	SE
3	0.132^{a1}	0.458^{ab1}	0.649^{ab1}	1.098^{b1}	0.192
5	0.146^{a1}	0.487^{b1}	0.671^{b1}	0.746^{b2}	0.073
10	0.137^{a1}	0.135^{a1}	0.144^{a1}	0.131^{a3}	0.016
SE	0.003	0.149	0.170	0.072	

* Column values with the same numbers are not significantly different, row values with the same letters are not significantly different ($p<0.05$). SE = Standard error of means.

increase in G' while tan δ remained unchanged.

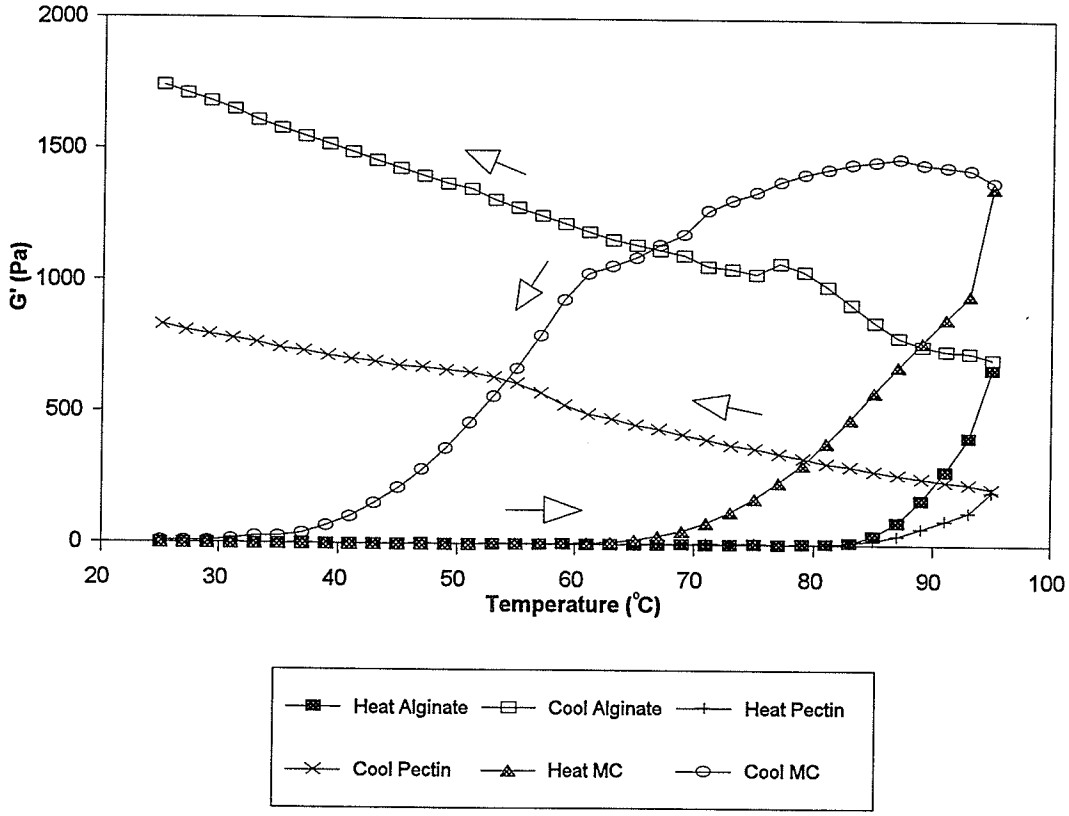

Figure 13. Effect of 1.5% various polysaccharides on the storage modulus G' of 5.0% BSA under heating and cooling phases of the rheological tests.

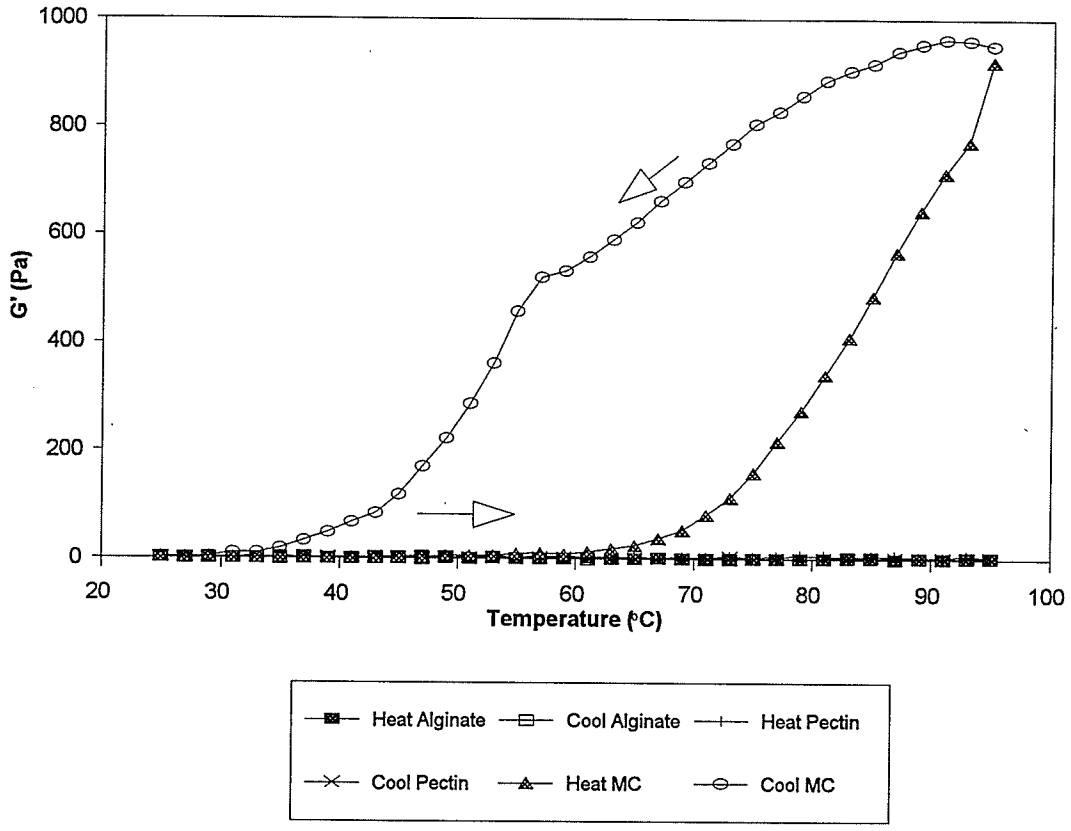

Figure 14. Storage modulus G' of 1.5% individual polysaccharides alone under heating and cooling phases of the rheological tests.

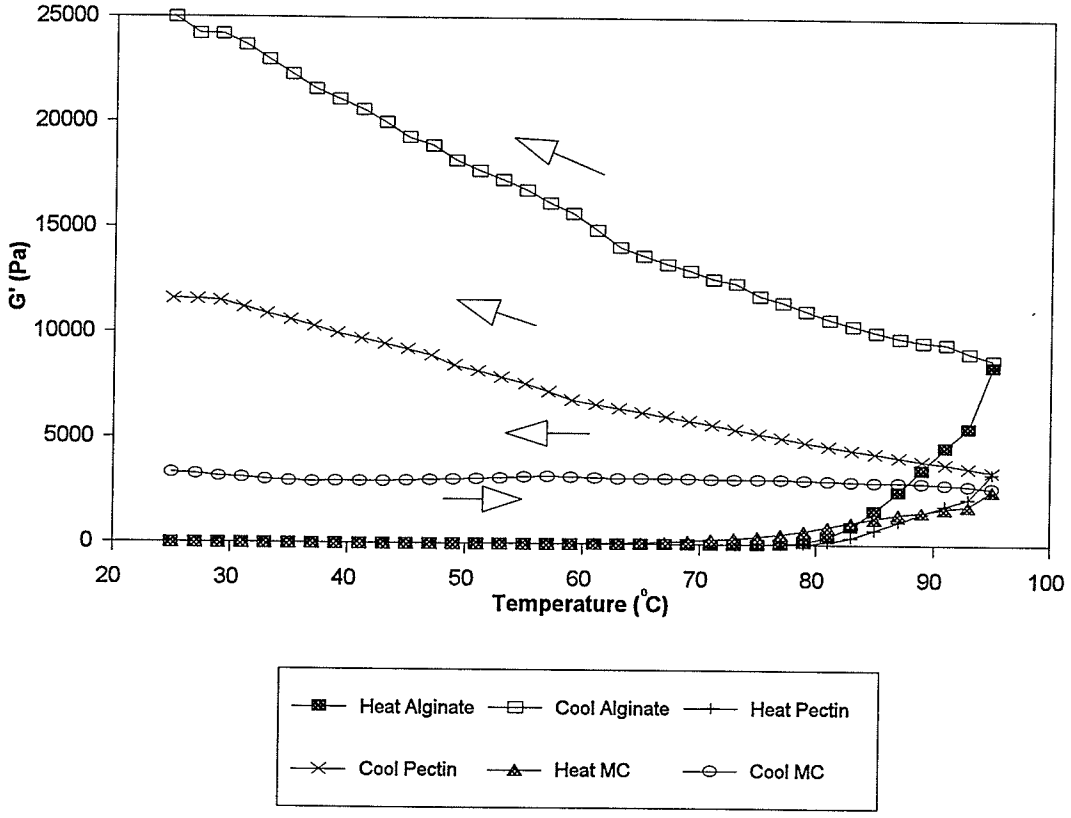

Figure 15. Effect of 1.5% various polysaccharides on the storage modulus G' of 10% BSA under heating and cooling phases of the rheological tests.

d. Overall Evaluation of Various BSA-Polysaccharide Systems

An overall effect of different protein and polysaccharide concentrations on gelation properties are shown in Tables 5 and 6. Overall evaluation was based on ANOVA data (Appendix 1) generated from statistical analysis of 3x3 randomized complete block design for each protein polysaccharide system. These ANOVA data results were expressed as main effect of protein concentration (Table 5) and main effect of polysaccharide concentration (Table 6). With the BSA-sodium alginate system, protein concentration had a significant effect on G' and tan δ. With an increase in concentration, an increase in G' and a decrease in tan δ resulted. With pectin systems, only a high protein concentration (10%) had a significant effect on G' (Table 5). Protein concentration had no effects on tan δ for the pectin systems. With methyl cellulose systems, a high protein concentration (10%) resulted in a significant increase in G' and a significant decrease in tan δ. Therefore, a better gel resulted at high protein concentrations in all three systems.

Polysaccharide concentrations also had significant effects on G' and tan δ (Table 6). With sodium alginate systems, high polysaccharide concentration (1.5%) resulted in a high G' value and a lower tan δ value, indicating a better gel resulted. With pectin systems, polysaccharide concentration had a significant effect on G' but had no effect on tan δ. With the methyl cellulose system, a high polysaccharide concentration (1.5%) resulted in a high G' and low tan δ value. However, the effect of polysaccharide concentration was found to be nonlinear in pectin systems. This was possibly related to the effect of binding ratio for the two biopolymers. In the case of the pectin systems, rheological testing was assessed at a pH value below the protein isoelectric point (Tables 5 and 6). As shown in turbidimetric analysis and gel filtration studies, binding between protein and anionic polysaccharides followed a particle binding model at this pH value.

Table 5. Effect of protein concentration on storage modulus G' (Pa) and tan δ of mixed BSA-polysaccharide systems at 1Hz generated from statistical analysis of 3x3 randomized complete block design.*

Systems	Protein Concentration (%) 3	5	10	SE
Effect on G' (Pa)				
Sodium Alginate[1]	199[a]	1040[b]	17365[c]	190
Pectin[2]	7[a]	105[a]	912[b]	45
Methyl Cellulose[3]	19[a]	41[a]	2272[b]	44
Effect on tan δ				
Sodium Alginate[1]	0.220[c]	0.138[b]	0.093[a]	0.009
Pectin[2]	0.351[a]	0.173[a]	0.142[a]	0.065
Methyl Cellulose[3]	0.735[b]	0.635[b]	0.143[a]	0.079

* Row values followed with the same letters were not significant different ($p<0.05$). All experiments were run in the natural pH of the mixtures. [1] pH=5.7, [2] pH=4.6, [3] pH=6.0. SE = Standard error of means.

In this binding model, the particle surface was saturated by the oppositely charged particle, therefore, protein macromolecules had difficulty to unfold, giving rise to poor gelation properties, since unfolding is a necessary step in heat induced gelation of globular proteins (Ferry, 1948).

The ANOVA data (Appendix 1) about interactions between the main effects of protein and polysaccharide were not given in forms of tables in the text. For all three systems, interactions between protein and polysaccharide had a significant effect on G' ($p<0.05$), but only BSA-sodium alginate had a significant effect on tan δ.

Table 6. Effect of polysaccharide concentration on storage modulus G' (Pa) and tan δ of mixed BSA-polysaccharide systems at 1Hz generated from statistical analysis of 3x3 randomized complete block design.*

Systems	Polysaccharide Concentration (%) 0.5	1.0	1.5	SE
Effect on G' (Pa)				
Sodium Alginate[1]	2405[a]	7064[b]	9135[c]	190
Pectin[2]	315[b]	120[a]	563[c]	45
Methyl Cellulose[3]	506[a]	383[a]	1443[b]	44
Effect on tan δ				
Sodium Alginate[1]	0.168[b]	0.146[ab]	0.136[a]	0.009
Pectin[2]	0.157[a]	0.224[a]	0.284[a]	0.065
Methyl Cellulose[3]	0.658[b]	0.488[ab]	0.366[a]	0.079

* Row values followed with the same letters were not significant different ($p<0.05$). All experiments were run in the natural pH of the mixtures. [1] pH=5.7, [2] pH=4.6, [3] pH=6.0. SE = Standard error of means.

B. Canola Protein - Polysaccharide Systems

1. Turbidimetric Analysis

Unlike BSA-anionic polysaccharide systems, 12S canola globulin-anionic polysaccharide systems did not readily form complexes at pH values immediately below the IEP (pH 7.2, Schwenke et al.,1987) of the 12S globulin, as shown in Fig.16. A turbidity change due to the presence of sodium alginate was found below pH 5.8. Unlike the control 12S curve which showed a decrease in turbidity after the pH value was brought to 3.6, the turbidity of 12S-sodium alginate system steadily increased with the decrease in pH value. This may suggest binding occuring at pH values well below the protein's isoelectric point. Furthermore, anionic polysaccharides seemed to play a co-soluble and stabilizing role for canola protein in the system at pH values near the isoelectric point, as shown in Table 7. Under unheated conditions, anionic polysaccharide systems have low turbidity, indicating better solubility than 12S alone or the neutral polysaccharide system. After the heat treatment, the turbidity of the anionic polysaccharide systems remained unchanged while that for neutral polysaccharide systems increased, suggesting that anionic polysaccharide may play a stabilizing role for the protein.

2. Gel Filtration

Methodology to further assess the 12S canola globulin-polysaccharide binding behaviour was similar to that used in BSA-polysaccharide systems. Changes have been made for heat treatment conditions and the pH value of the buffer. All combinations of

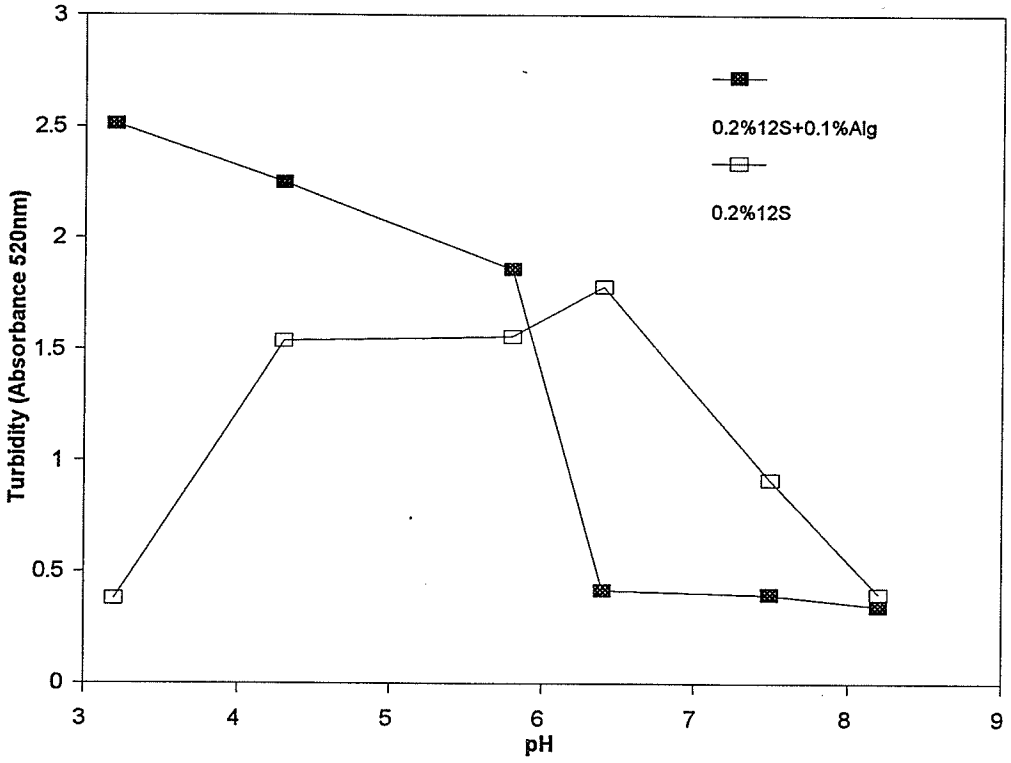

Figure 16. Turbidity of canola 12S globulin-anionic polysaccharide in relation to pH values.

Table 7. Turbidity (520 nm) of 12S canola globulin-polysaccharide systems under unheated and heated conditions.

Systems	Unheated (pH 7.4)	Heated (pH 6.4)	SE
0.2% 12S + 0.1% alginate	0.35[a]	0.35[a]	0.07
0.2% 12S + 0.1% pectin	0.30[a]	0.35[a]	0.05
0.2% 12S	1.40[a]	1.35[a]	0.21
0.2% 12S + 0.1% MC	1.60[a]	1.75[b]	0.07
0.2% 12S + 0.1% guar gum	1.65[a]	1.75[b]	0.07

Row values with the same letters are not significantly different ($P<0.05$). SE = Standard error of means.

12S canola globulin and polysaccharides were run in a 0.1 M sodium phosphate buffer at pH 8.8. This value was close to pH 9.0 that was used in the rheological test. The pH and ionic strength of the buffer were chosen to avoid interference of salt in the protein-polysaccharide interactions while keeping the pH as close as possible to the one used in rheological testing so that binding behaviour could be related to the rheological test. Concentrations used for the unheated systems were 1% 12S canola protein and 0.5% polysaccharide. Lower concentrations of 0.2% 12S protein and 0.1% polysaccharide were used for heated systems due to sample precipitation at higher concentrations. The precipitation would make the protein impossible to elute through the column, since precipitate would stop on the top of the column. Lower sample concentrations prevented protein precipitation in the presence of polysaccharide when heat was applied.

Heat treatments for 12S canola globulin and polysaccharide systems were

conducted at 90 °C for 5min. Since the denaturation temperature of canola protein isolate was found to be around 80 °C (Léger and Arntfield, 1993), the temperature chosen was thought to be sufficient to cause the protein denaturation necessary for unfolding and possibly interacting with polysaccharides, if present.

The 12S canola globulin used for gel filtration, which has been fractionated from canola protein isolate, was the major component (about 60% of total protein; Wong, 1995) of the canola protein isolate used in the rheological test. After fractionation, the 12S globulin fraction had a protein content of 95% in comparison with 79% in the canola protein isolate. Fractionation of the 12S canola globulin facilitated the binding study between protein and polysaccharides since binding behaviour is easier to assess with a single fraction.

a. Sodium Alginate and Pectin Systems

Unheated samples of mixtures of 12S globulin and polysaccharide showed similar nonbinding behaviour as the BSA-polysaccharide systems. No binding was found under unheated conditions at pH 8.8, as shown in Fig.17. However, there was a disturbance to the 12S peak in the elution profile of each system. This may be attributed to the increase in viscosity of the eluent in the presence of polysaccharides. As shown in Fig.17, the elution profile of the 12S globulin alone showed a major 12S component and a low molecular weight component. The elution profile of 12S in the mixture exhibited a slight peak shift for both 12S and low molecular weight fractions. The peaks were also broader. This may result from higher viscosities for the systems in the presence of polysaccharides. The peak of sodium alginate was well separated from that of the 12S globulin in the mixture, indicating there was no binding in that system. Under heating conditions, as shown in Fig.18, the peak for the low molecular weight subunit of protein

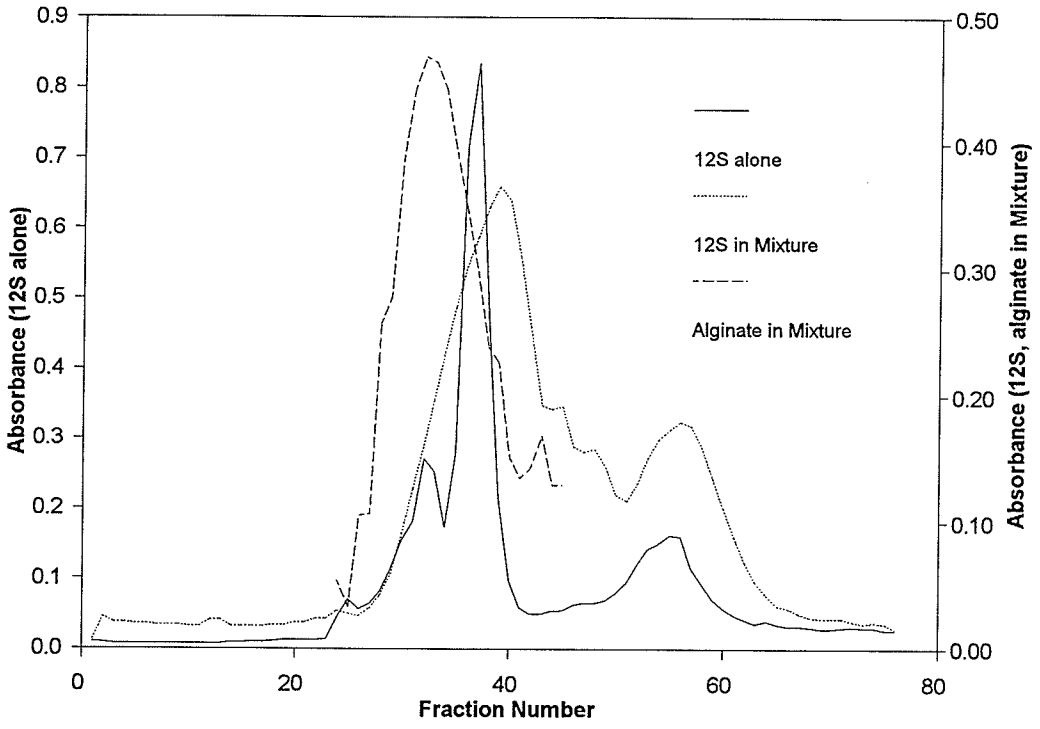

Figure 17. Elution profile of 12S canola globulin alone and 12S-sodium alginate mixture unheated. Sample size: 5.0 mL of 1.0% 12S alone, 5.0 mL of 1.0% 12S and 0.5% sodium alginate. Each fraction had a volume of 8.0 mL. Eluent: 0.1 M phosphate buffer, pH 8.8.

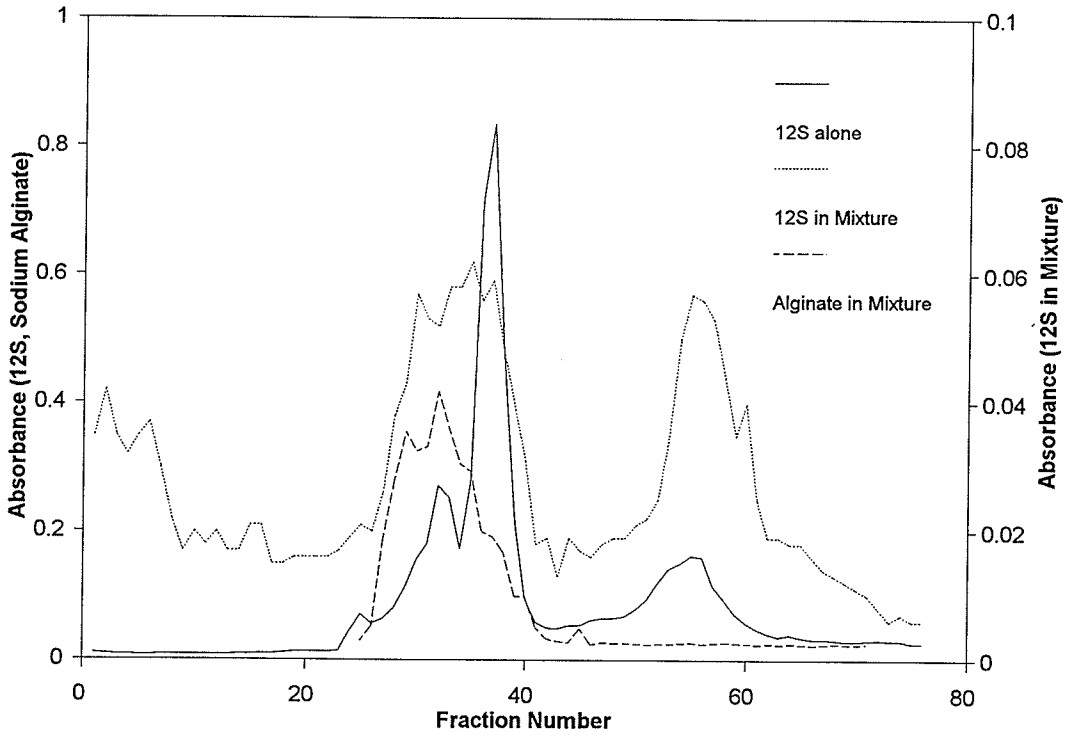

Figure 18. Elution profile of 12S canola globulin alone and 12S-sodium alginate mixture after heat treatment. Sample size: 5.0 mL of 1.0% 12S alone, 10.0 mL mixture of 0.2% 12S and 0.1% sodium alginate heated at 90°C for 5min. Each fraction had a volume of 8.0 mL. Eluent: 0.1 M phosphate buffer, pH 8.8.

was more pronounced at the expense of the 12S component, indicating there was an increase in the amount of subunit and a decrease in 12S content after heat treatment. This was obviously attributed to the heat decomposition of the 12S globulin, since higher ration of lower molecular weight fraction was observed in the elution profile of 12S canola globulin-sodium alginate mixture after heat treatment (Fig. 18). It was suspected that the heat instability of the 12S globulin had been in some way responsible for the nonbinding behaviour with polysaccharide and consequently for its gelation properties in food systems. Although a slight shift in peak position of the 12S component was seen, the change was not pronounced. The signals for main peak of 12S globulin and alginate in the mixture were not equivalent (Fig. 18). Therefore, the observed results were not sufficient to indicate a binding behaviour between the two biopolymers. As seen in turbidimetric analysis, sodium alginate seemed to play a stabilizing role for the 12S canola globulin. It prevented 12S globulin from denaturing. If this was the case, the broad peak for the 12S globulin at low concentrations of the mixed polymers could be explained by partial denaturation of some protein causing a shift towards the void volume while others remained at the same position due to the stabilizing effect of sodium alginate. On the other hand, no binding was found between the low molecular weight fraction and sodium alginate.

The elution profile of the pectin-12S canola globulin mixture resembled that of sodium alginate-12S canola globulin system, as shown in Fig.19. A marked peak shift towards a higher elution volume was also observed. However, the low molecular weight fraction was distributed and not evident in this system. The shift of the peak towards the higher elution volume may also be attributed to the increase in viscosity in the presence of pectin.

After the heat treatment, as shown in Fig. 20, high absorbance in the low

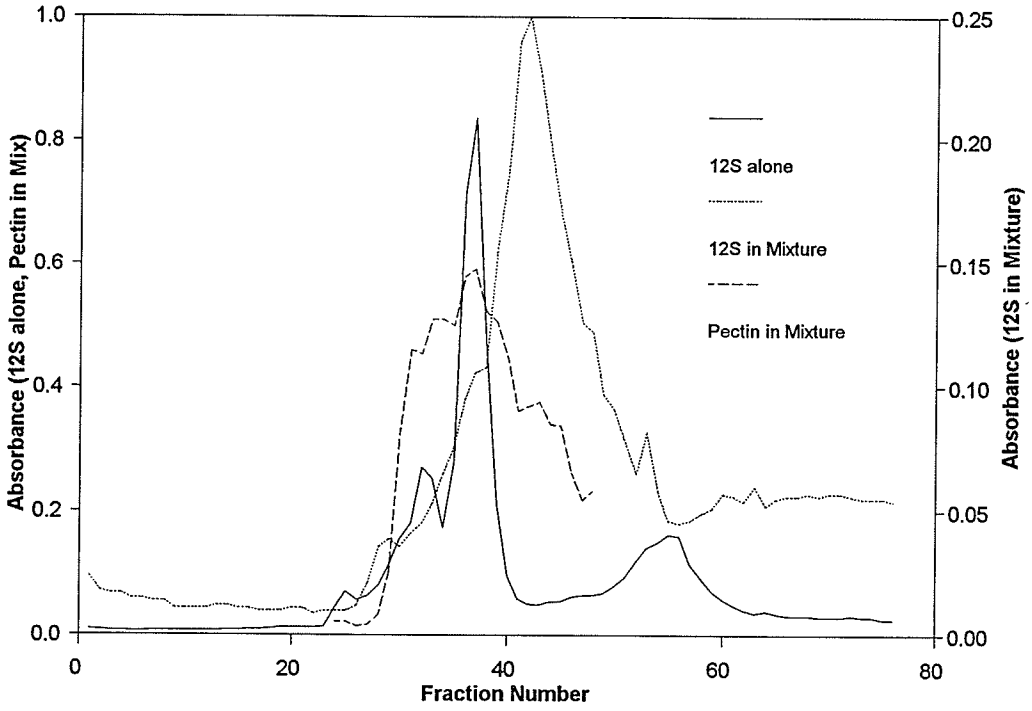

Figure 19. Elution profile of 12S canola globulin alone and 12S-pectin mixture unheated. Sample size: 5.0 mL of 1% 12S alone, 5.0 mL mixture of 1.0% 12S and 0.5% pectin. Each fraction had a volume of 8.0 mL. Eluent: 0.1 M phosphate buffer, pH 8.8.

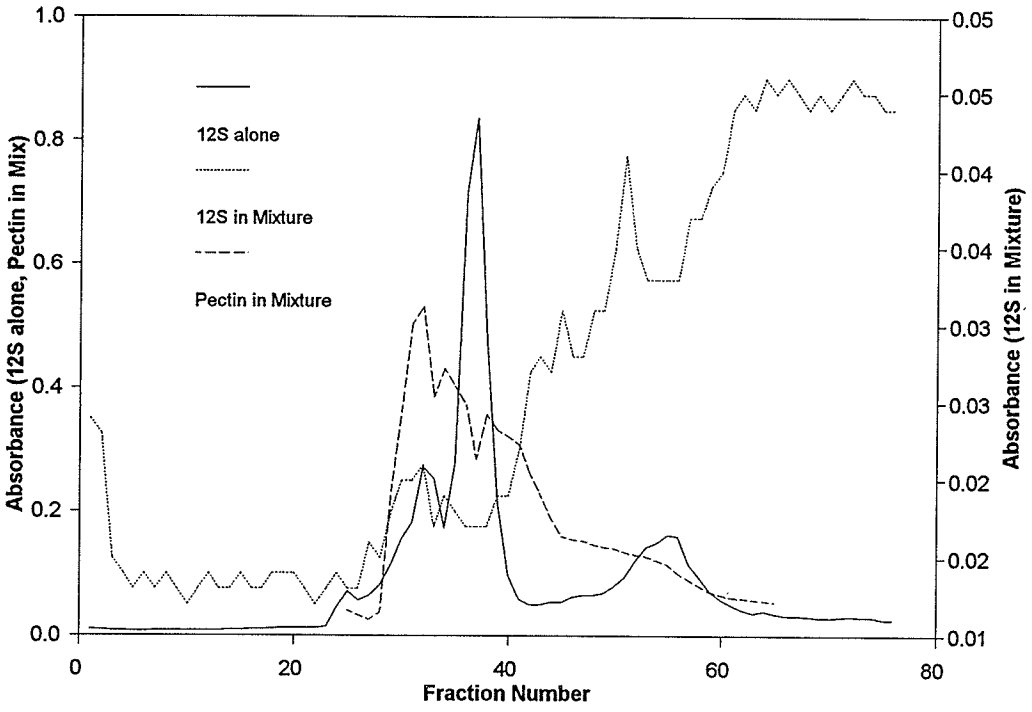

Figure 20. Elution profile of 12S canola globulin alone and 12S-pectin mixture after heat treatment. Sample size: 5.0 mL of 1.0% 12S alone, 10.0 mL mixture of 0.2% 12S and 0.1% Pectin heated at 90°C for 5min. Each fraction had a volume of 8.0 ml. Eluent: 0.1 M phosphate buffer, pH 8.8.

molecular weight subunit region was observed, while the peak of the 12S component nearly completely disappeared. Only a small peak showed up at a lower fraction volume corresponding to the 12S component. This may be related to a denatured 12S component. A high amount of low molecular weight fraction resulted from the heat decomposition of 12S canola globulin, behaviour similar to that observed in sodium alginate system but a much stronger decomposition action was seen here in the pectin system. No binding was observed. This was probably due to the heat decomposition of the 12S canola globulin.

b. Methyl Cellulose Systems

Elution profiles of the mixture of the 12S globulin and methyl cellulose exhibited similar spectral properties (Fig. 21). No binding between 12S and methyl cellulose was found. Slight peak shifts were also due to the increase in viscosity due to the presence of methyl cellulose. It was impossible to perform a gel filtration profile for heated mixture of 12S globulin and methyl cellulose due to the protein precipitation in the sample upon heating.

3. Visual Assessment

Similar to the BSA-polysaccharide systems, visual assessment is important in determining the compatibility between the biopolymers for the systems. Samples from turbidimetric analysis and gel filtration were assessed visually. At low concentrations, namely 0.2% 12S and 0.1% polysaccharide, the 12S globulin was soluble and appeared clear in the presence of anionic polysaccharides at pH values close to the IEP (pH 7.2, Schwenke et al., 1987) of the 12S canola globulin (Table 8). In the presence of neutral polysaccharide, on the other hand, the system appeared cloudy due to the insoluble

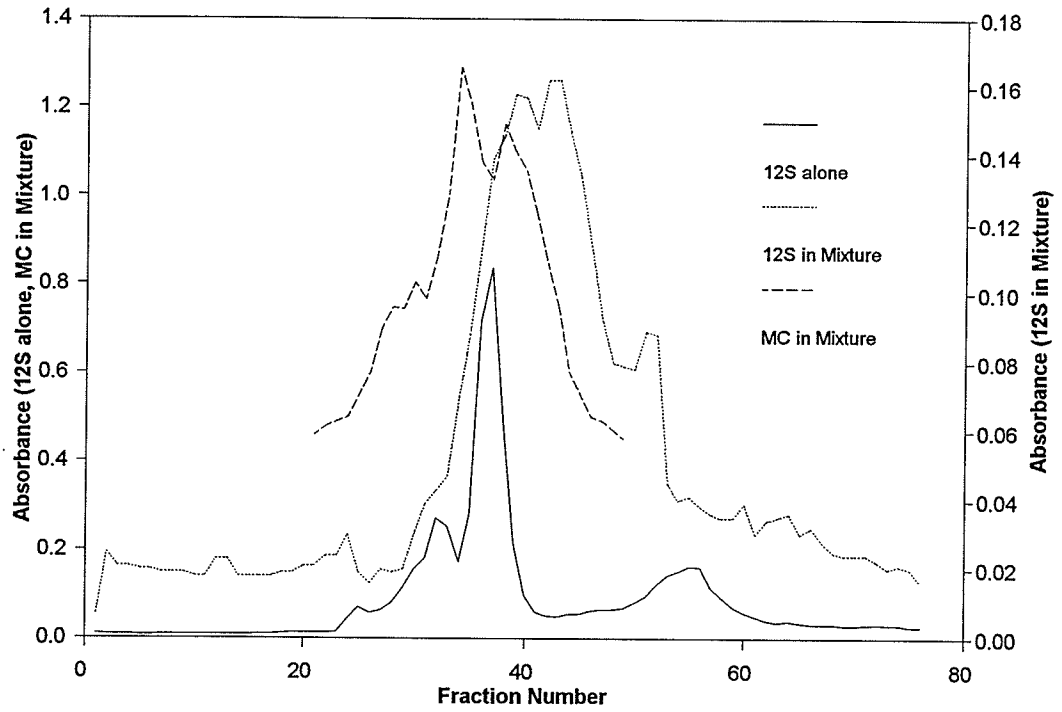

Figure 21. Elution profile of 12S canola globulin alone and 12S-methyl cellulose (MC) mixture unheated. Sample size: 5.0 mL of 1.0% 12S alone, 5.0 mL mixture of 1.0% 12S and 0.5% methyl cellulose. Each fraction had a collection of 8.0 ml. Eluent: 0.1 M phosphate buffer, pH 8.8.

Table 8. Results of visual assessment at different conditions for 12S canola globulin-polysaccharide systems for turbidimetric analysis and gel filtration and canola protein isolate-polysaccharide systems for rheological test.

Systems	Conditions					
	turbidity (0.3% 12S)		pH 8.8 for gel filtration (0.3%12S)		pH 9.0 for rheology (5-15% isolate)	
	pH7.2	pH6.4	unheated	heated 90°C,5 min	before test	resulting gel
Alginate	clear	clear	clear	clear	viscous liquid	slurry or opague gel
Pectin	clear	clear	clear	clear	viscous liquid	slurry or opaque gel
Methyl Cellulose	cloudy	cloudy	clear	cloudy	2 phase	slurry or opaque gel

property of the 12S globulin at pH values near IEP of the protein. The presence of anionic polysaccharides influenced the electrostatic layers surrounding the protein molecules by introducing extra repulsive force between molecules. This may account for the high solubility and the clear appearance of the systems. The pH seemed to not play a dominant role for the binding behaviour of the biopolymers, as it was in BSA-anionic polysaccharide systems. By adjusting the pH value away from protein isoelectric point towards basic side, the solubility of the 12S globulin in the methyl cellulose system increased. As a result, the system become soluble and appeared clear at more basic pH conditions. After heat treatment, the systems of the 12S globulin and mixed 12S globulin-

methyl cellulose became cloudy even under basic pH conditions due to the denaturation of the 12S globulin while systems of 12S globulin-anionic polysaccharide remained clear. The phenomenon may be explained by the stabilizing effect of the anionic polysaccharide through electrostatic interaction (Imeson et al., 1977) compared to no electrostatic effect for the neutral polysaccharide systems. In the latter, therefore, the behaviour of the system appeared to represent a combination of the behaviour of individual components and therefore appeared cloudy, that means 12S globulin in a neutral polysaccharide system performed similarly to 12S globulin alone.

Canola protein isolate prepared for rheological testing was also assessed visually. Before the rheological testing, two phases were noted for the canola protein isolate-methyl cellulose and -guar gum systems. Only one phase was observed for canola protein isolate-sodium alginate and -pectin systems. Mixtures were agitated to be as homogeneous as possible before applied to the Bohlin Rheometer. After the rheological testing, there were no apparently visual differences among the resulting gels from different systems.

4. Rheology

a. Effect of pH

A search for the best pH conditions for gelation studies led to the investigation of the effect of different pH values on the gelation properties. Results are presented in Table 9. The highest G' values were generally found at pH values away from neutral. At acidic conditions, however, G' values were accompanied by high tan δ values, indicating the gel was probably strengthened through aggregation rather than network formation since gel formed by aggregation was less structured (higher tan δ) than by three dimension network (Ma and Holme, 1982). At high pH values, tan δ values were

Table 9. Effect of pH on storage modulus G' (Pa) and tan δ of the mixed canola protein isolate-polysaccharide gels at 1 Hz.*

Isolate (%)	pH	control	1.0%alginate	1.0%pectin	SE
Effect on G'					
15	4.5	961^{c}	147^{a}	578^{b}	65
	6.2	525^{b}	237^{a}	357^{a}	32
	9.0	575^{b}	241^{a}	262^{a}	27
	10.0	815^{c}	349^{a}	514^{b}	58
Effect on Tan δ					
15	4.5	0.195^{a}	0.351^{b}	0.258^{a}	0.025
	6.2	0.194^{a}	0.198^{a}	0.201^{a}	0.008
	9.0	0.114^{a}	0.149^{b}	0.104^{a}	0.010
	10.0	0.089^{a}	0.126^{b}	0.099^{a}	0.008

*Row values with the same letters are not significantly different ($P<0.05$).
SE = Standard error of means.

low, indicating good gel network formation. The pH value for this study was therefore set at pH 9.0. A comparison among mixed canola protein isolate-anionic polysaccharide at different pH conditions showed that the detrimental effect of anionic polysaccharide on the gelation of canola protein occurred in all pH regions (Table 9).

b. Sodium Alginate Systems

Rheological data of mixed canola protein isolate-sodium alginate are given in Tables 10a-b. The tests were conducted in 0.1 M NaCl at pH 9.0. Isolate concentration played a dominant role in determining gel strength and elasticity. Both properties improved with an increase in isolate concentration in both control sample and in mixed systems, as shown in Tables 10a-b. The effect of isolate concentration on storage

Tables 10a-b. Rheological results of (a) storage modulus G' (Pa) and (b) tan δ for mixed canola protein isolate-sodium alginate gel at 1 Hz.*

(a)

Isolate (%)	Sodium Alginate (%)				
	control	0.5	1.0	1.5	SE
5	8^{a1}	9^{a1}	9^{a1}	11^{a1}	3
10	66^{c2}	32^{b2}	28^{b1}	12^{a1}	3
15	190^{b3}	178^{b3}	142^{ab2}	93^{a2}	15
SE	3	5	16	5	

(b)

Isolate (%)	Sodium Alginate (%)				
	control	0.5	1.0	1.5	SE
5	0.086^{a1}	0.258^{a1}	0.294^{a1}	0.287^{a1}	0.079
10	0.113^{a1}	0.087^{a1}	0.144^{a1}	0.222^{a1}	0.042
15	0.104^{a1}	0.106^{a1}	0.133^{b1}	0.218^{c1}	0.003
SE	0.014	0.073	0.053	0.048	

* Column values with the same numbers are not significantly different, rows with the same letters are not significantly different ($p<0.05$). SE = Standard error of means.

modulus during gelation is shown in Fig. 22. Increasing protein isolate concentration resulted in an increase in G'. Surprisingly, in contrast to the BSA-sodium alginate

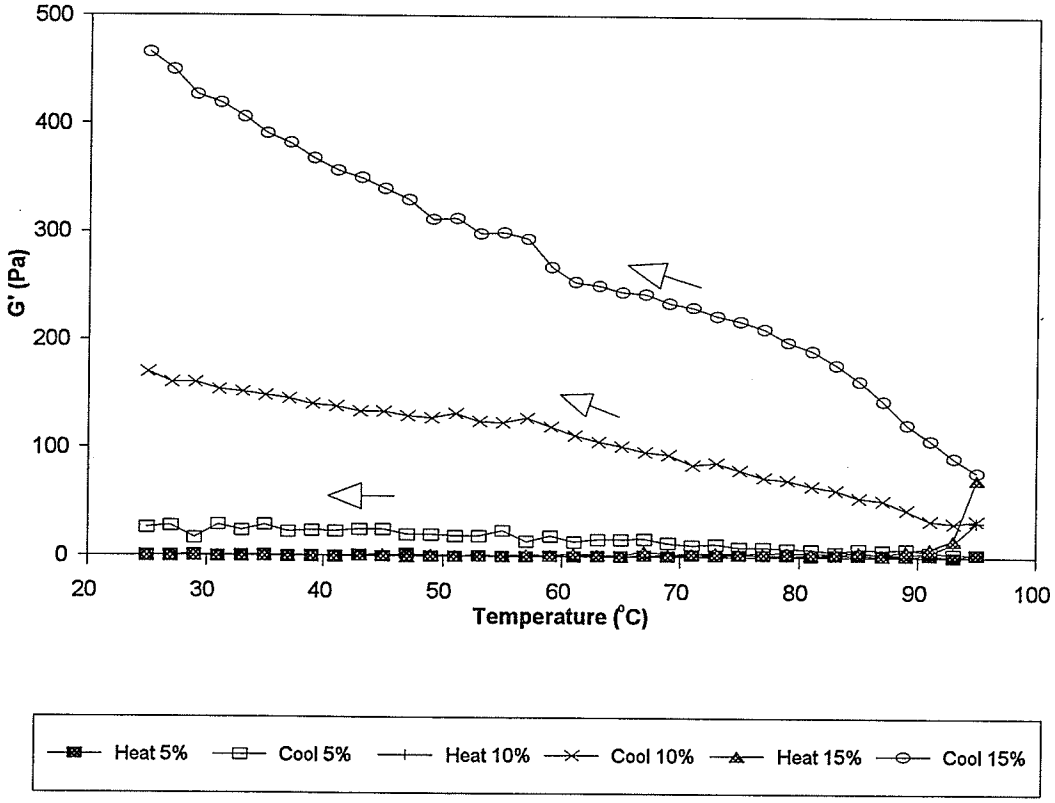

Figure 22. Storage modulus G' of canola protein isolate in relation to different concentrations under heating and cooling phases of the rheological test.

system, sodium alginate had a significantly detrimental effect on both G' and tan δ. At 15% isolate concentration, with increasing sodium alginate concentration, there was a significant decrease in G' and a significant increase in tan δ (Tables 10a-b). The gel filtration profile revealed that there was no binding between the 12S canola globulin and sodium alginate at pH 8.8. In addition, the 12S globulin was readily broken down into lower molecular weight fraction upon heating at 90 °C. It was suspected whether the detrimental effect of sodium alginate on the gelation properties of canola protein could be attributed to the enhancement of aggregation through the masking of charge repulsion by the electrostatic interaction with sodium alginate, since masking charge repulsion demonstrated previously with sodium chloride could lead to poor gelation properties (Arntfield, 1990c). Detrimental effects of sodium alginate were also seen during the course of gelation (Fig.23).

The detrimental effect was more significant in the presence of 0.1 M NaCl than without salt, as shown in Table 11.

The detrimental effect of salt on the protein gelation properties has been investigated previously. It has been suggested that very low concentrations of salts aid in protein solubilization prior to heating and provide a cross-link in the network (Kohnhorst and Mangino, 1985; Mulvihill and Kinsella, 1988). There is a point, however, where the masking of the net charge repulsion is the dominant factor and further salt addition simply promotes aggregation. In an investigation of ovalbumin and vicilin, the deterioration of ovalbumin networks began at NaCl concentrations greater than 0.3 M, while for vicilin a similar effect was seen at concentrations greater than 0.2 M (Arntfield et al., 1990c). Only a slightly detrimental effect was seen at salt concentrations less than these values. In this study, the effect of 0.1 M NaCl on the G' and tan δ of mixed canola protein-polysaccharide gel showed a similar network

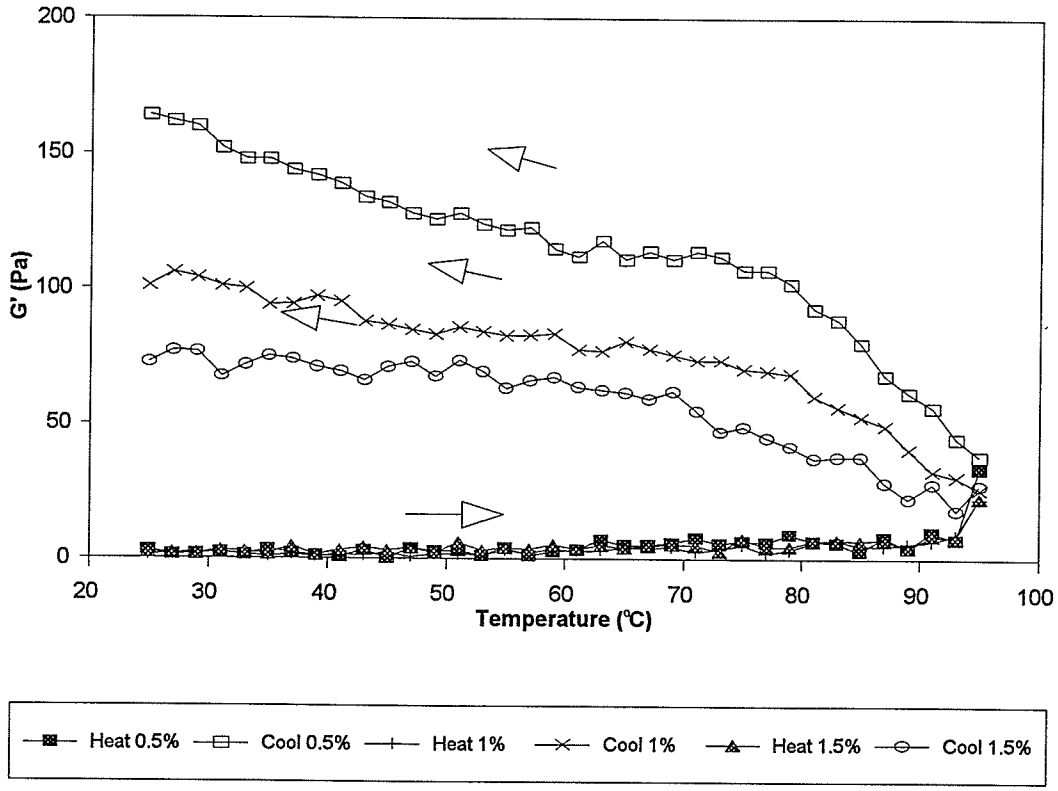

Figure 23. Effect of sodium alginate concentration on the storage modulus G' of 15% canola protein isolate under heating and cooling phases of the rheological tests in the presence of 0.1M NaCl.

Table 11. Effect of 0.1M NaCl on the storage modulus G' (Pa) and tan δ of mixed canola protein isolate-polysaccharide systems at 1 Hz.*

canola isolate (%)	alginate (%)	0.1M NaCl	0M NaCl	SE
Effect on G'(Pa)				
10	0.5	32[a]	115[b]	3
10	1.0	28[a]	46[b]	2
15	1.5	93[a]	238[b]	7
Effect on tan δ				
10	0.5	0.087[a]	0.088[a]	0.023
10	1.0	0.144[b]	0.090[a]	0.002
15	1.5	0.218[b]	0.147[a]	0.004

*Row values with the same letters are not significantly different ($P<0.05$).
SE = Standard error of means.

deterioration pattern. Salt concentration was low, however, compared to the previous data for ovalbumin and vicilin. The masking of protein charge repulsion by salt and therefore the promotion of protein aggregation is believed to be the major reason for the detrimental effect.

c. Pectin Systems

Rheological data of mixed canola protein isolate-pectin are given in Tables 12a-b. The effect of pectin on the rheological properties of canola protein was conducted at pH 9.0 without salt. The effect of pectin was also found to be detrimental to network formation. G' values of mixed canola protein isolate-pectin gels were generally lower than the control sample. Though G' values were proportional to the pectin content in the

Tables 12a-b. Rheological results of (a) storage modulus G' (Pa) and (b) tan δ for mixed canola protein isolate-pectin gel at 1 Hz*

(a)

Isolate (%)	Pectin (%)				
	control	0.5	1.0	1.5	SE
5	30^{a1}	36^{ab1}	35^{ab1}	39^{b1}	2
10	179^{b2}	107^{a2}	134^{ab2}	158^{ab12}	14
15	575^{b3}	179^{a3}	265^{a3}	265^{a2}	31
SE	12	8	12	35	

(b)

Isolate (%)	Pectin (%)				
	control	0.5	1.0	1.5	SE
5	0.110^{a1}	0.116^{a12}	0.121^{a12}	0.122^{a1}	0.015
10	0.111^{a1}	0.152^{a2}	0.147^{a2}	0.145^{a1}	0.011
15	0.114^{ab1}	0.102^{a1}	0.114^{ab1}	0.130^{b1}	0.006
SE	0.017	0.009	0.006	0.008	

* Column values with the same numbers are not significantly different, rows with the same latters are not significantly different ($p<0.05$). SE = Standard error of means.

system, most of these values were found to be not significantly different. Within the three levels of pectin (0.5, 1.0, 1.5%), the pectin concentration was found to have no

significant effect on G' except when the isolate was at a concentration of 5%. Tan δ values were found to increase significantly only at high isolate concentrations. These results indicated there was an increase in gel elasticity and at the same time a decrease in three dimensional network of the gel as the pectin concentration in the system increased. Therefore, higher G' values at higher pectin concentrations were attributed to promoting aggregation in the presence of pectin rather than the formation of a three dimensional network (Tables 12a-b).

The detrimental effect of anionic polysaccharides on canola protein isolate gelation may be attributed to several factors. Deterioration through electrostatic interactions upon introducing anionic polysaccharides may be a factor, since similar effects were not seen in a mixed protein isolate-neutral polysaccharide systems, as will be discussed later. This effect would be important as binding behaviour was not seen for the 12S globulin-anionic polysaccharide systems based on gel filtration. Molecular inhomogeneity of the system may also be a factor. The 12S protein was readily broken down upon heating. The inhomogeneity of the system increased with the addition of polysaccharides and became pronounced after the heating phase of gel formation due to heat decomposition of the protein. The molecular inhomogeneity may inhibit the interaction among the adjacent chains in the system and prevent the formation of a three dimension network, since the more efficiently these interactions in forming junction zones or bundles are dispersed in the sol, the stronger is the network that is subsequently formed (Rees, 1969). In addition, the role of electrostatic interactions by masking the net charge repulsion demonstrated by the effect of salt on protein gelation (Arntfield et al., 1990c) may have similar effect in the presence of sodium alginate and be responsible for the increase in the rate of aggregation during the heating phase. The protein anionic polysaccharide systems in this study were considered to be thermodynamically compatible since no phase separation was

observed.

d. Methyl Cellulose Systems

Results of the rheological data for the mixed canola protein isolate-methyl cellulose system were interesting as gelation properties were improved at low protein concentrations (Tables 13a-b). A significant increase in G' was observed at a concentration of 5% canola isolate with an increase in methyl cellulose concentration. At high isolate concentration (15%), however, the effect was detrimental. Although the results at high protein concentration were similar to these for the BSA-methyl cellulose system, the results at low protein concentrations were quite different. Tan δ values were not significantly changed at low system concentrations. At high protein concentration, tan δ increased. This indicated a less elastic gel at high protein concentrations but gel elasticity was maintained at low protein concentrations.

The effect of methyl cellulose on the gelation of the mixed canola protein isolate-methyl cellulose system is shown in Fig.24. The stages of gelation during heating and cooling followed the gelation pattern of MC in general (Fig.14). The interesting point was the system retained much more of the gel consistency at room temperature than pure methyl cellulose or BSA-methyl cellulose systems. This indicated an improvement in the gelation properties has been achieved at low protein concentration. Unlike the BSA-methyl cellulose system, where the hydrophobic interactions of methyl cellulose were promoted by the addition of BSA to the system, in canola protein isolate-methyl cellulose system, the hydrophobic interactions of methyl cellulose at high temperature were disturbed with the addition of canola protein, as reflected in low G' values during gelation.

Tables 13a-b. Rheological results of (a) storage modulus G' (Pa) and (b) tan δ for mixed canola protein isolate-methyl cellulose gel at 1 Hz.*

(a)

Isolate (%)	Methyl Cellulose (%)				
	control	0.5	1.0	1.5	SE
5	30^{a1}	19^{b1}	88^{c2}	99^{d3}	2
10	179^{b2}	32^{a1}	11^{a1}	37^{a2}	10
15	575^{b3}	16^{a1}	1^{a1}	21^{a1}	5
SE	12	4	4	1	

(b)

Isolate (%)	Methyl Cellulose (%)				
	control	0.5	1.0	1.5	SE
5	0.110^{a1}	0.280^{b3}	0.170^{a1}	0.173^{a1}	0.017
10	0.111^{a1}	0.171^{b1}	0.317^{d1}	0.295^{c2}	0.005
15	0.114^{a1}	0.223^{a2}	0.719^{b2}	0.290^{a2}	0.070
SE	0.017	0.007	0.080	0.009	

* Column values with the same numbers are not significantly different, rows with the same latters are not significantly different ($p<0.05$). SE = Standard erro of means.

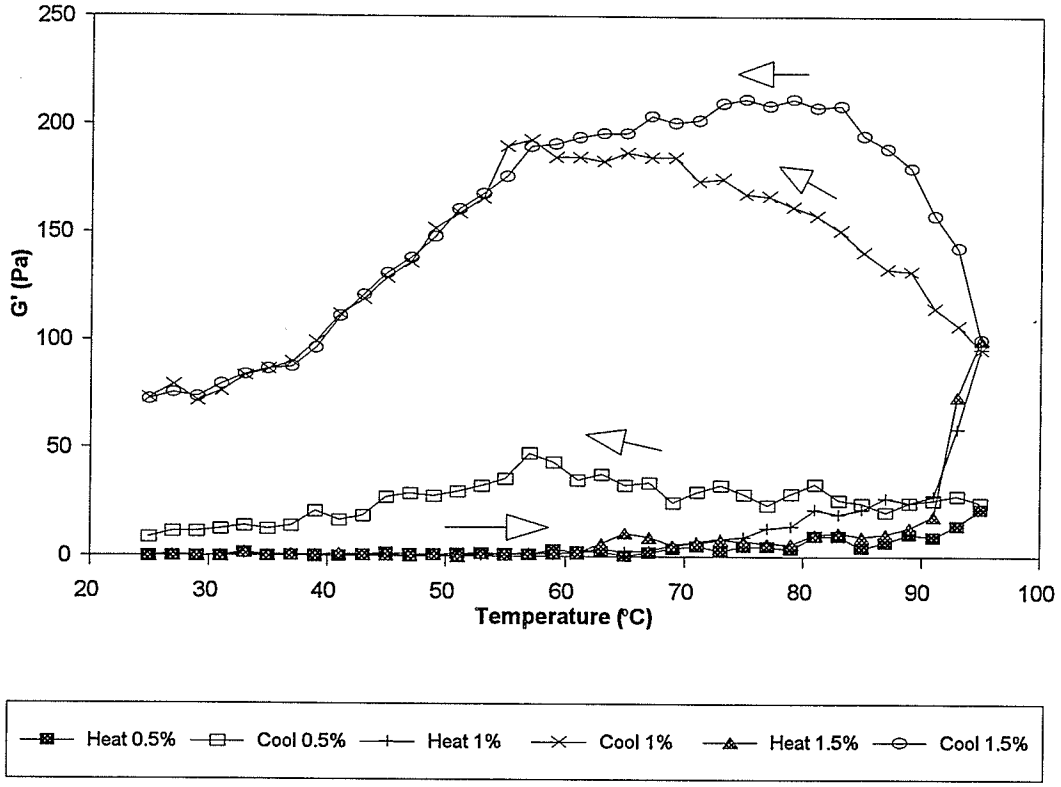

Figure 24. Effect of methyl cellulose concentration on the storage modulus G' of 5% canola protein isolate under heating and cooling phases of the rheological tests at 1Hz.

e. Guar Gum Systems

Guar gum was used in this study to get more insight into the concept that neutral polysaccharides have a synergistic effect on protein gelation as seen in the mixed canola protein isolate-methyl cellulose system. The results are shown in Tables 14a-b. With an increase in polysaccharide concentration, there was a significant increase in G' at low isolate concentrations (5%, 10%). At high isolate concentration, only the G' value at 1.5% polysaccharide concentration was found to be significantly different from the others. Isolate concentration, on the other hand, had a significant effect on G' values for all series. Tan δ values, however, were also found to significantly increase with the increase in concentrations of both protein and polysaccharide. This means network formation was weakened (high tan δ) with the increase in gel elasticity (high G'). It confirmed that neutral polysaccharides had an effect on the gelation of canola isolate. The biopolymer systems were incompatible since two separate phases were observed. The synergistic effect of the neutral polysaccharides may arise from the local interactions between chain fragments of the same polymer. This may lead to some separated network regions resulting in the construction of the entire mixed gel in the system (Ziegler and Foegeding, 1990). An increase in concentration led to an increase in system incompatibility, which eventually appeared to destroy the balance required for a mixed gel composed of small networks.

The mechanism of mixed gel formation seemed to largely depend on the type of neutral polysaccharides. In the case of pure methyl cellulose, hydrophobic interactions were the dominant forces in gel formation. At high temperatures, gel structure was promoted. Gel structure was weakened when the temperature decreased. It was found that the mixed canola protein-methyl cellulose gel retained strong gel structure after the temperature returned to room temperature as compared to the rheological curve of pure

Tables 14a-b. Rheological results of (a) storage modulus G' (Pa) and (b) tan δ for mixed canola protein isolate-guar gum gel at 1 Hz.*

(a)

Isolate (%)	Guar Gum (%)				
	control	0.5	1.0	1.5	SE
5	30^{a1}	68^{b1}	139^{c1}	206^{d1}	7
10	179^{a2}	280^{b2}	420^{c2}	459^{c12}	10
15	575^{ab3}	465^{a3}	621^{ab3}	768^{b2}	60
SE	12	10	5	69	

(b)

Isolate (%)	Guar Gum (%)				
	control	0.5	1.0	1.5	SE
5	0.110^{a1}	0.199^{b3}	0.344^{c3}	0.292^{c3}	0.015
10	0.111^{a1}	0.151^{b2}	0.177^{c2}	0.242^{d2}	0.004
15	0.114^{a1}	0.137^{ab1}	0.141^{ab1}	0.161^{b1}	0.007
SE	0.017	0.002	0.004	0.009	

* Column values with the same numbers are not significantly different, rows with the same letters are not significantly different ($p<0.05$). SE = Standard error of means.

methyl cellulose. This phenomenon was not seen in BSA-methyl cellulose system. In addition, the hydrophobic interactions of mixed gel were not as strong as in pure methyl

cellulose gel.

With guar gum, it seemed that hydrogen bonds were the dominant forces for gelation, since hydration could be very strong and rapid in guar gum solution (Whistler and Hymowitz, 1979), which may provide beneficial H-bonding in the system. The beneficial synergistic effect of guar gum was once again demonstrated in a comparison of the effect of low concentration (0.1%) of polysaccharides on gelation properties of 10% canola protein gel, as shown in Fig.25.

f. Overall Evaluation of Various Canola Protein Isolate-Polysaccharide Systems

An overall effect of different protein and polysaccharide concentrations on the gelation properties of various mixed systems is shown in Tables 15 and 16. Overall evaluation was based on the ANOVA data (Appendix 1) generated from statistical analysis of 3x3 randomized complete block design for each protein-polysaccharide system. The results were expressed as the main effect of protein concentration (Table 15) and the main effect of polysaccharide concentration (Table 16). Overall assessments can be seen for each polysaccharide system. For the anionic polysaccharides, the sodium alginate system (in 0.1M NaCl) showed very weak gelation properties even with protein concentrations as high as 15% and G' values decreased with an increase in sodium alginate content (Table 16). The pectin system (no salt) showed a significant increase in G' with the increase of either protein (Table 15) or pectin concentration (Table 16). There was a significant difference in tan δ values at different protein concentrations, but there was no significant difference for different polysaccharide concentrations.

With the neutral polysaccharides, there was a significant increase in G' at low protein concentrations (Table 15) and high methyl cellulose concentrations (Table 16). Tan δ values were also significantly lower in these conditions. In the case of guar gum,

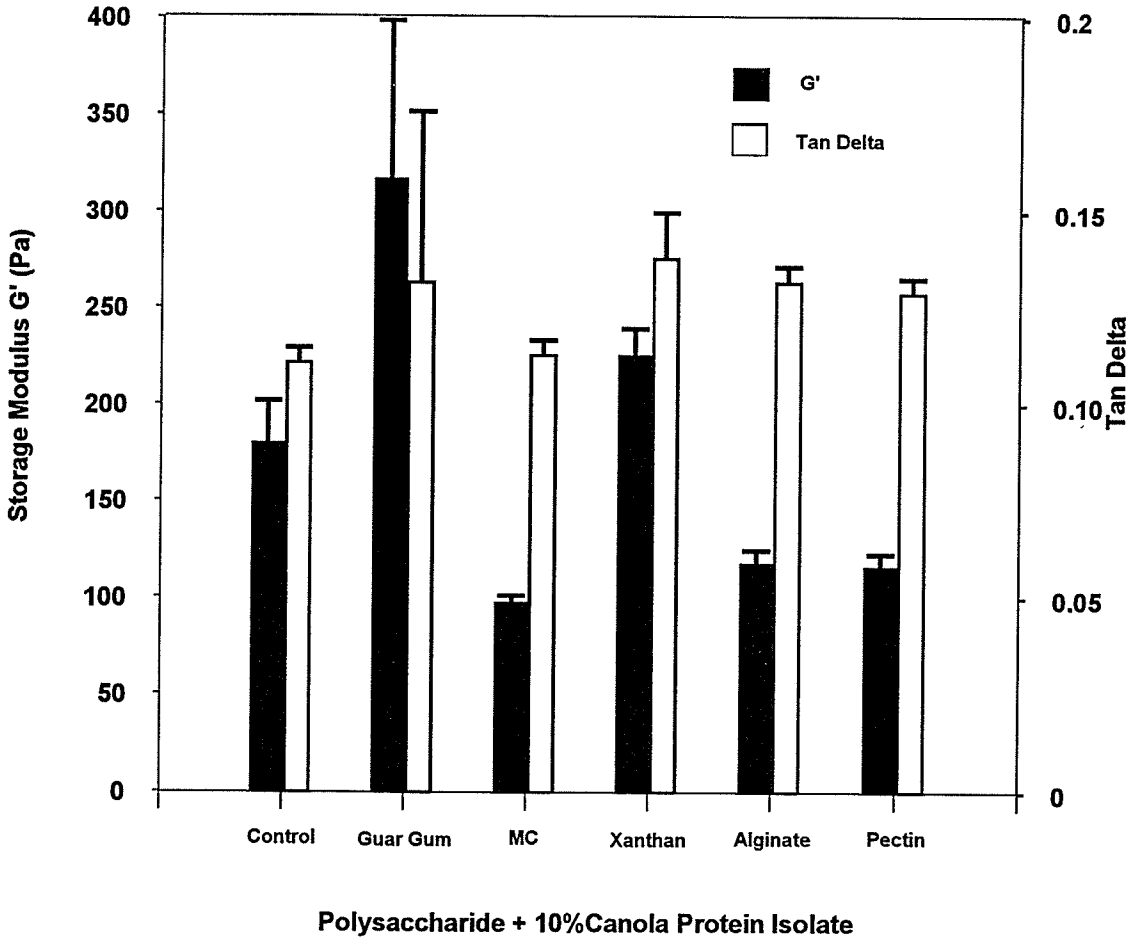

Figure 25. Effect of 0.1% various polysaccharides on the storage modulus G' and tan δ of 10% canola protein isolate at 1Hz.

Table 15. Effect of protein concentration on storage modulus G' (Pa) and tan δ of mixed canola protein isolate-polysaccharide systems at 1 Hz generated from statistical analysis of 3x3 randomized complete block design.*

Systems	Protein Isolate Concentration (%) 5	10	15	SE
Effect on G' (Pa)				
Sodium Alginate	10^a	24^a	138^b	6
Pectin	37^a	133^b	236^c	12
Methyl Cellulose	68^a	27^b	14^c	2
Guar Gum	137^a	386^b	618^c	23
Effect on tan δ				
Sodium Alginate	0.280^a	0.153^b	0.151^b	0.034
Pectin	0.120^a	0.148^b	0.115^a	0.005
Methyl Cellulose	0.208^a	0.261^a	0.411^b	0.027
Guar Gum	0.278^a	0.190^b	0.146^c	0.003

* Row values with the same letters are not significantly different ($p < 0.05$).
SE = Standard error of means.

G' significantly increased with the increase of either protein (Table 15) or guar gum concentration (Table 16). Tan δ values were decreased with the increase in protein concentration but increased with the increase of guar gum concentrations.

The ANOVA data (Appendix 1) about interactions between the main effects of protein and polysaccharide were not given in forms of tables in the text. Interaction between canola protein isolate and sodium alginate and the interaction between canola protein isolate methyl cellulose were found to have a significant effect on G', while the interaction between protein isolate and methyl cellulose and the interaction between

Table 16. Effect of polysaccharide concentration on storage modulus G' (Pa) and tan δ of mixed canola protein isolate-polysaccharide systems at 1 Hz generated from statistical analysis of 3x3 randomized complete block design.*

Systems	Polysaccharide Concentration (%) 0.5	1.0	1.5	SE
Effect on G' (Pa)				
Sodium Alginate	73[a]	60[a]	39[b]	6
Pectin	107[a]	145[ab]	154[b]	12
Methyl Cellulose	22[a]	35[b]	52[c]	2
Guar Gum	271[a]	393[b]	477[c]	23
Effect on tan δ				
Sodium Alginate	0.150[a]	0.190[a]	0.243[a]	0.034
Pectin	0.124[a]	0.127[a]	0.132[a]	0.005
Methyl Cellulose	0.225[a]	0.402[b]	0.253[a]	0.027
Guar Gum	0.162[a]	0.221[b]	0.232[c]	0.003

* Rows values with the same letters are not significantly different ($p<0.05$).
SE = Standard error of means.

isolate and guar gum were found to have a significant effect on tan δ.

V. DISCUSSION

A. BSA-Polysaccharide Systems

1. Thermodynamically Compatible Systems

Within the concentration range in this study, systems are considered to be thermodynamically compatible for both anionic polysaccharide systems, namely BSA-sodium alginate and BSA-pectin. The nature of the compatible system lies in the binding behaviour of the biopolymers. Complexes form upon mixing of the biopolymers under certain conditions, eg. pH values below the IEP or pH values above the IEP when the mixture was subjected to a heat treatment. Binding mechanisms were attributed to the electrostatic attraction existing between the oppositely charged macromolecules in general. There are however, some difference in binding pathways for different thermodynamic conditions, such as different pH values and temperatures. At pH values below the IEP, electrostatic interactions may occur between two globoids having the opposite charge. The binding ratio depends on the relative intensity of the charge on the globular surfaces. The interaction among the whole unit of the macromolecules results in the association of a large colony and subsequently precipitation, namely the insoluble complexes. Because of the formation of large macromolecular complexes, unfolding of the peptide chain becomes difficult, therefore, the formation of gel networks is impossible. These results were shown in the turbidimetric analysis in Fig.3 and rheological results in Fig. 12 for the anionic polysaccharide systems at pH values below the IEP. For a compatible system it can be concluded that complexes formed by the

stabilization of electrostatic attractions at pH value below the IEP result in poor rheological properties. Unable to unfold the peptide chain or to promote localized entanglement of these peptide chains may account for the detrimental effect on the development of a gel network. Binding in this case was suggested by this thesis to be a particle model since it happened between particles having opposite charges, which has been defined as non-specific ionic interaction in a previous research (Stainsby, 1980).

The binding pathway at pH values above the IEP presents a different model from the previous one. Binding does not happen under natural conditions, but only occurs after heating, as demonstrated by the gel filtration elution profiles. This means binding was associated with some alternation in protein molecular structure due to heat. The logical conclusion was related to the denaturation of the macromolecules. Unfolding of the macromolecular chains caused the exposure of some net positive charge in a micro-region of the peptide chain (Imeson et al., 1977). It is suspected that the stabilization mechanism is still electrostatic in nature, though there may be some covalent components. A comparison between sodium alginate and pectin systems shows there are some things that were different. The unusual heat stability of sodium alginate-BSA complex and its translucent appearance may indicate some covalent interactions are involved, although there are only reports on esters of alginate-BSA systems being dependent on the covalent binding mechanism (Stainsby, 1980). This possibility needs to be confirmed by further experimentation. Pectin complexes, on the other hand, exhibited cloudy appearances upon heating and had limited heat stability, a phenomenon similar to the complex formed at pH values below the IEP. The complex precipitated at temperatures above 80 °C and was thought to be less stable than sodium alginate which did not precipitate under the same condition. In the rheological testing, similar binding through heat induced unfolding and electrostatic and/or covalent bond formation should be expected in the heating phase of

the rheological test. Binding in this case was suggested by this thesis to be a chain segment model, since interaction could only occur between positive charged groups in polypeptide chain and negative charged groups in polysaccharide (Imeson et al., 1977; Stainsby, 1980).

The above binding pathway results in a significant increase in gel elasticity (G') and a good three dimension network (tan δ). These conclusions were based on gel filtration diagrams and rheological data. Binding in some areas or micro-regions results in a complex unit, which will not prevent the unfolding and entanglement of the biopolymers. Micro-region binding branches could contribute more entanglement and therefore, the system exhibited a significant synergistic benefit.

A comparison between the above two thermodynamically compatible systems results in the conclusion that the gelation properties are largely dependent on the thermodynamic conditions. Gelation was promoted at pH values above the IEP but suppressed at pH values below the IEP for anionic polysaccharide system, suggesting there are differences in conformational potential, which has been described as the ability of the protein to form intermolecular junction zones that contribute to the desired structural properties (Tolstoguzov, 1992). Below the IEP there is a low conformation potential and above the IEP a high conformational potential. The difference originates from the lack of the ability to unfold and entangle in the former, while conformational potential in the latter is enhanced through attachment of a limited number of branches on the macromolecules. The thermodynamic properties of the system reflect a change in conformational potential caused by thermodynamic parameters such as pH and temperature. These suggestions were supported by the results in Fig. 12, which show the effect of pH values below and above the IEP on the rheological data.

The binding ratio was not found to be a critical factor in determining the

properties of the BSA-sodium alginate gel. Instead, it was the concentrations of the complexes which had a significant effect. Low concentrations of the complex resulted in a weaker gel (lower G' and higher tan δ) in comparison with control BSA (Table 2a). On the other hand, high concentration of the complex resulted in a significant increase in G' and decrease in tan δ. An example is seen in Table 2a, where doubling the concentration and keeping the ratio constant, 5:0.5 and 10:1, resulted in an increase in G' from 231 to 19900 Pa, and a decrease of tan δ from 0.173 to 0.093, namely a better gel formed at higher complex concentrations. This may suggest that the complex behaviour was distinct from that of polysaccharide and protein. The initial concentration of these copolymers required for gelation was higher than that for BSA alone. This may be attributed to the difficulty of connection in establishing junction zones when the peptide chains were interrupted by polysaccharides. In other words, sodium alginate will contribute a beneficial synergistic effect only at BSA concentration above 5%. Details of the mechanism need more investigation.

The translucent appearance of BSA-sodium alginate needs to be noted for its speciality from the other anionic polysaccharides, i.e. pectin, at similar pH values. This may be beneficial when incorporating it into a food system.

2. Thermodynamically Incompatible Systems

The gel filtration data for the methyl cellulose system indicated there was no binding behaviour in the system, neither in unheated, nor in heated conditions. However, gel filtration alone does not give a justification for thermodynamic incompatibility. Visual assessment provides the complementary information. Phase separation was noted in all combinations of BSA-methyl cellulose system, meaning the systems are incompatible at the stated concentrations, which may be attributed to the incompatible nature of the

biopolymers. The properties of the system resulted in a combination of rheological properties of the individual biopolymers. At low BSA concentrations, the mixture exhibited properties similar to those for methyl cellulose whereas at high concentrations, the rheological properties reflected the BSA component. Nevertheless, methyl cellulose contributed to a limited extent a beneficial synergistic effect to BSA gelation properties. The conclusion in this case was that an incompatible system resulted in combined rheological properties where each component performed in its natural way. The contribution of methyl cellulose to a high concentration BSA system may attributed to a filled agent effect on G' (Ziegler and Foegeding, 1990). Tan δ was found to be not significantly different from the control BSA.

B. Canola Protein-Polysaccharide Systems

1. Thermodynamically Compatible Systems

As shown in the turbidimetric analysis, gel filtration and visual assessment, there was no binding between the 12S globulin and anionic polysaccharides at pH 8.8 either before or after heat treatment. No cloudiness was observed at this pH value after heating. In addition, at the intermediate pH region, the mixed 12S globulin-anionic polysaccharide systems appeared to be soluble, probably due to the co-soluble effect involving electrostatic interactions. No phase separation was observed under the concentrations investigated. These systems were therefore considered compatible. However, no evidence of complex formation was obtained at pH 8.8. Unlike BSA-anionic polysaccharide systems, the anionic polysaccharide had a detrimental effect on canola protein gel structure. Nonbinding behaviour and heat decomposition sensitivity may account for the difficulty in developing three dimensional networks for this protein. In addition, the

influence of electrostatic interactions associated with charged groups in anionic polysaccharides masked the charge repulsive force and encouraged aggregation, thereby giving rise to an aggregated mass rather than a gel network. The detrimental effect seemed stronger with sodium alginate than with pectin. The presence of 0.1 M NaCl in the biopolymer systems appeared to strengthen this detrimental effect. This phenomenon agreed with the previous study done on vicilin but revealed a low critic concentration values of salt for this system (Arntfield et al., 1990c).

A comparison of canola protein-anionic polysaccharide systems with BSA-anionic polysaccharide systems led to the conclusion that thermodynamically compatible system could, but may not necessarily, improve gelation properties at pH values above protein IEP. However, these systems do have their own gelation patterns. With the BSA systems, binding under heat treatment accounted for the significant improvement in gelation properties. With canola protein, there was no binding. The electrostatic masking through charged group may be the dominant factor responsible for the detrimental effect to the gel structure provided charge influence by sodium alginate was similar to the influence by sodium chloride, which has been demonstrated in many systems (Arntfield et al., 1990c; Kohnhorst and Managino, 1985; Mulvihill and Kinsella, 1988). Heat decomposition and possibly less flexibility for the canola protein may be responsible for the nonbinding behaviours with charged polysaccharides since binding in this case was based on the exposure of charged group in some micro-regions of polypeptide chain (Imeson et al., 1977; Stainsby, 1980). Heat decomposition may destroy the local charged structure.

2. Thermodynamically Incompatible Systems

Similar to BSA systems, canola protein isolate-neutral polysaccharide systems

were considered incompatible since a two phase separation was observed under the concentrations investigated. Cloudiness and high values of absorbance in the turbidimetric analysis may be attributed to the low solubility of the 12S globulin at these pH conditions. Neutral polysaccharide may have no effect at this point, as revealed by the absorbance data for both the control and mixed systems. The gelation properties of incompatible canola protein isolate-polysaccharide systems, like the BSA systems, exhibited properties representing a combination of its individual components. However, unlike the BSA systems, in which the deterioration of gel structure seemed not to depend on the extent of incompatibility, with canola isolate system, as the incompatibility increased as a result of increasing polymer concentrations, the gelation properties were affected. In the case of methyl cellulose, the nature of the incompatible systems at high concentrations resulted in detrimental effects in terms of gel structure. With guar gum, improvements in gelation properties were seen at all concentrations but they were not as high in high concentrations as in low concentrations of the total biopolymers. Unlike BSA, no significant improvement in gelation properties was obtained at high canola protein concentration. However, the improvement by neutral polysaccharide at low canola concentration could be of potential importance in canola protein utilization.

VI. CONCLUSIONS AND RECOMMENDATIONS

A. Conclusions

1. Systems containing mixtures of proteins and anionic polysaccharides, namely sodium alginate and pectin, were thermodynamically compatible within the concentrations studied, 3-10% of BSA, 5-15% of canola protein isolate and 0.5-1.5% of anionic polysaccharide. The compatible system of BSA-polysaccharides resulted in binding under heat treatment at pH values above protein IEP through a chain segment model, giving rise to an improvement in gelation properties. The compatible 12S globulin-anionic polysaccharide systems did not form complexes under heating conditions at pH above the protein's IEP. This may account for the nonbinding behaviour. The masking of repulsive forces by introducing the charged groups of anionic polysaccharide may account for the detrimental effect of polysaccharide on canola protein gelation.

2. Gelation properties of compatible systems are unique, different from either individual component. These properties are largely dependent on the substances involved, binding models and the thermodynamic conditions. In the BSA-polysaccharide systems, gelation properties were promoted at pH values above the protein's IEP but suppressed at pH below the IEP. This was attributed to the network improvement by chain segment binding between protein and anionic polysaccharide. In canola protein isolate-polysaccharide systems, gelation properties deteriorated at pH values both above and below the protein IEP.

3. Protein-neutral polysaccharide systems were thermodynamically incompatible.

In both BSA- and canola protein-polysaccharide systems, phase separation resulted at concentration as low as 3% BSA, 5% canola protein and 0.5% polysaccharide. The incompatibility increased with the increase in biopolymer concentrations.

4. Gelation properties of thermodynamically incompatible systems exhibited, in most cases, the combined properties of the individual components. With the BSA system, a beneficial synergistic effect of polysaccharides on the gels was observed at high BSA (10%) concentrations. In canola protein isolate systems, the combined effects of the mixed components produced stronger gels than canola protein alone at low protein concentrations. At high protein concentrations, however, incompatibility increased, giving rise to structure disruption in the canola protein isolate-methyl cellulose system. In guar gum systems, at high biopolymer concentrations, mixed gel properties improved, but not as much as at low protein concentrations where the extent of incompatibility was lower.

B. Recommendations

1. In BSA-anionic polysaccharide systems, whether or to what extent the binding ratio has an effect needs more investigation, since the ratio would appear to be a factor affecting gelation properties of the complexes.

2. In BSA-sodium alginate systems, whether binding involves covalent or noncovalent interactions needs further clarification. This could be done by investigating the role of covalent bond in the system with or without the addition of noncovalent bond disrupting agent, such as urea.

3. Details on nonbinding behaviour of canola protein-anionic polysaccharides needs more close investigation, since a binding behaviour similar to that of BSA should

also occur at pH values above the protein's IEP, provided canola protein performed similarly to BSA upon heating.

4. Detrimental mechanism of anionic polysaccharide on the gelation properties of canola protein isolate needs more investigation.

5. Remarkable changes in gelation properties were only seen in protein-anionic polysaccharide. As seen in BSA system, these changes were related to the thermodynamic conditions of the system. The question of whether there is a possibility that under some certain conditions a chain segment binding between 12S and anionic polysaccharide can also occur should be further investigated.

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VIII. APPENDICES

APPENDIX 1

ANOVA data of the rheological result G' and tan δ of protein-polysaccharide systems based on 3x3 randomized complete block designs.

Table A1. ANOVA of BSA-sodium alginate systems.

Dependent Variable: G'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1484324471	185540559	859.15	0.0001
Error	9	1943631	215959		
Total	17	1486268102			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BSA	2	1123759802	561879901	2601.79	0.0001
ALG	2	142565469	71282735	330.08	0.0001
BSA*ALG	4	217999200	54499800	252.36	0.0001

Dependent Variable: tan δ

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.06094000	0.00761750	15.90	0.0002
Error	9	0.00431050	0.00047894		
Total	17	0.06525050			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BSA	2	0.04950233	0.02475117	51.68	0.0001
ALG	2	0.00323633	0.00161817	3.38	0.0804
BSA*ALG	4	0.00820133	0.00205033	4.28	0.0327

Table A2. ANOVA of BSA-pectin systems.

Dependent Variable: G'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	5027686.177	628460.772	51.74	0.0001
Error	9	109325.304	12147.256		
Total	17	5137011.482			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BSA	2	2961975.750	1480987.875	121.92	0.0001
PEC	2	586857.161	293428.581	24.16	0.0002
BSA*PEC	4	1478853.266	369713.316	30.44	0.0001

Dependent Variable: tan δ

Source	DF	Sum of quares	Mean Square	F Value	Pr > F
Model	8	0.33101944	0.04137743	1.62	0.2433
Error	9	0.22981700	0.02553522		
Total	17	0.56083644			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BSA	2	0.15249211	0.07624606	2.99	0.1012
PEC	2	0.04871078	0.02435539	0.95	0.4210
BSA*PEC	4	0.12981656	0.03245414	1.27	0.3498

Table A3. ANOVA of BSA-methyl cellulose systems.

Dependent Variable: G'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	32475501.95	4059437.74	348.75	0.0001
Error	9	104760.61	11640.07		
Total	17	32580262.56			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BSA	2	20097605.05	10048802.53	863.29	0.0001
MC	2	4028217.29	2014108.64	173.03	0.0001
BSA*MC	4	8349679.61	2087419.90	179.33	0.0001

Dependent Variable: tan δ

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	1.70908400	0.21363550	5.68	0.0088
Error	9	0.33826400	0.03758489		
Total	1	2.04734800			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
BSA	2	1.20525633	0.60262817	16.03	0.0011
MC	2	0.25868033	0.12934017	3.44	0.0776
BSA*MC	4	0.24514733	0.06128683	1.63	0.2488

Table A4. Canola protein isolate-sodium alginate systems.

Dependent Variable: G'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	66827.10111	8353.38764	41.78	0.0001
Error	9	1799.49500	199.94389		
Total	17	68626.59611			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CAN	2	59064.59111	29532.29556	147.70	0.0001
ALG	2	3620.08111	1810.04056	9.05	0.0070
CAN*ALG	4	4142.42889	1035.60722	5.18	0.0192

Dependent Variable: tan δ

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.09914700	0.01239338	1.77	0.2068
Error	9	0.06310950	0.00701217		
Total	17	0.16225650			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CAN	2	0.06554433	0.03277217	4.67	0.0406
ALG	2	0.02572033	0.01286017	1.83	0.2147
CAN*ALG	4	0.00788233	0.00197058	0.28	0.8830

Table A5. Canola protein isolate-pectin systems.

Dependent Variable: G'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	132243.9644	16530.4956	17.86	0.0001
Error	9	8330.4000	925.6000		
Total	17	140574.3644			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CAN	2	119650.2711	59825.1356	64.63	0.0001
PEC	2	7325.2311	3662.6156	3.96	0.0585
CAN*PEC	4	5268.4622	1317.1156	1.42	0.3024

Dependent Variable: tan δ

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.00466844	0.00058356	4.40	0.0202
Error	9	0.00119450	0.00013272		
Total	17	0.00586294			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CAN	2	0.00380211	0.00190106	14.32	0.0016
PEC	2	0.00024478	0.00012239	0.92	0.4322
CAN*PEC	4	0.00062156	0.00015539	1.17	0.3856

Table A6. Canola protein isolate-methyl cellulose systems.

Dependent Variable: G'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	18016.8786	2252.10983	101.51	0.0001
Error	9	199.67250	22.18583		
Total	17	18216.55111			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CAN	2	9608.008611	4804.004306	216.53	0.0001
MC	2	2718.777778	1359.388889	61.27	0.0001
CAN*MC	4	5690.092222	1422.523056	64.12	0.0001

Dependent Variable: tan δ

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.46353344	0.05794168	13.04	0.0004
Error	9	0.03997900	0.00444211		
Total	17	0.50351244			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CAN	2	0.13331344	0.06665672	15.01	0.0014
MC	2	0.10870878	0.05435439	12.24	0.0027
CAN*MC	4	0.22151122	0.05537781	12.47	0.0010

Table A7. Canola protein isolate-guar gum Systems.

Dependent Variable: G'

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	839561.0000	104945.1250	32.56	0.0001
Error	9	29006.1250	3222.9028		
Tota	17	868567.1250			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CAN	2	693427.5833	346713.7917	107.58	0.0001
GUA	2	129493.0833	64746.5417	20.09	0.0005
CAN*GUA	4	16640.3333	4160.0833	1.29	0.3432

Dependent Variable: tan δ

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	8	0.08533344	0.01066668	166.23	0.0001
Error	9	0.00057750	0.00006417		
Total	17	0.08591094			

Source	DF	Type I SS	Mean Square	F Value	Pr > F
CAN	2	0.05426711	0.02713356	422.86	0.0001
GUA	2	0.01680078	0.00840039	130.92	0.0001
CAN*GUA	4	0.01426556	0.00356639	55.58	0.0001