# ENHANCED GELATION OF FIELD PEA PROTEINS THROUGH FORMATION OF MULTICOMPONENT SYSTEMS USING VARIOUS POLYSACCHARIDES

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Tamara Ranadheera

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**FACULTY OF GRADUATE STUDIES**

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**Tamara Ranadheera**

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

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# ABSTRACT

The potential for enhanced gelation of globular plant proteins through the inclusion of food-grade polysaccharides was established experimentally for pea protein isolate in combination with either locust bean gum, guar gum or κ-carrageenan. Both factorial and response surface statistical designs were constructed to screen, optimize and verify physicochemical factors significantly contributing to the gelation of these mixed systems. Design factors included protein concentration, protein to polysaccharide ratio, protein to salt ratio and pH. Evaluation of the elastic (G') and storage (tan δ) modulus, acquired from small amplitude oscillatory rheological testing, was used to characterized the resulting networks. Behavior of the bipolymer systems were additionally considered through differential scanning calorimetry and solubility assessment. The addition of guar gum and carrageenan resulted in comparable improvements in pea protein gelation. Improved gelation was not evidenced by the interaction of these polysaccharides with pea protein but rather by their incompatibility within solution. Results based on graphical and numerical optimization showed that protein-guar gum systems displayed well-defined gel networks at pHs closer to pea protein's IEP. At a pH of 5.32, protein concentrations could vary anywhere between 11.59 and 28.41% while maintaining protein-polysaccharide ratios below 60.63. Carrageenan improved pea protein gelation at higher alkaline pHs (ie. pH>7.70). In such systems however, protein levels above 13.9% and protein-polysaccharide ratios less than 41.30 were necessary. As such when developing a favorable gel from a composite system, guar gum systems demonstrated more flexibility and less restriction in terms of physiochemical parameters (i.e. protein and polysaccharide levels).

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# I. INTRODUCTION

The blending of two or more materials in an endeavor to attain a unique property not possessed by the original components, is a common practice. Commonly observed applications of polymer composites with improved mechanical traits include rubber tires, fiberglass and flexible packaging. Previous research has demonstrated that it can be economically beneficial to substitute, at least partially, a high-priced gelling agent with inexpensive ones if product functionality is not diminished (Aguilera 1992). This has led to recent investigations into the synergistic interactions and added functionality of multicomponent food systems (Ledward 1994; Samant et al. 1993; Tolstoguzov 1995).

Proteins are commonly utilized in food systems to bring forth increased functionality such as gelling, thickening and emulsion stabilization. However, such functional proteins often occur in food systems, where polysaccharides, possessing their own functional roles, are also present. A great deal of research has been devoted to the study of both proteins and polysaccharides. However the majority of attention has focused primarily on the behavior of each macromolecule individually. Therefore, corresponding to the growing trend in research (Tolstoguzov 1995, 1998), the present investigation examines the mutual occurrence of proteins and polysaccharides in a model system and the significance of their interactions.

The growing practice of supplementing proteins with polysaccharides in order to improve functionality has been used in conjunction with enhancing protein emulsifying properties (Ledward 1979; Kiosseoglou and Doxastakis 1988; Tolstoguzov et al. 1985). These effects have been attributed to the formation of electrostatic complexes between

proteins and polysaccharides (Tolstoguzov et al. 1985). However, only recently has the research broadened to encompass the effects of polysaccharides upon other functional properties of food proteins such as gelation (Ziegler and Foegeding 1990; Xiong and Blanchard 1993 and DeFreitas et al. 1997). Protein gelation is achieved through a balance of protein-protein and protein-solvent interactions, under which a continuous three-dimensional network evolves. The phenomenon of globular protein gelation from seed storage proteins will be the focus of this study. Recent investigations related to their functionality, have increased the incentive to develop food proteins from plant origins (Paulson and Tung 1989; Bacon et al. 1990). In addition, the high nutritional value and potential availability of such proteins makes them of interest to the food industry, especially in the area of human consumption (Burova et al. 1992). Field pea protein was used in this investigation to assess a typical globular protein's performance in association with polysaccharides. Locust bean gum and guar gum were chosen to represent neutral polysaccharides while κ-carrageenan illustrated the influence of an anionic polysaccharide.

A closer examination of protein-polysaccharide systems appears to be pertinent in view of the expanding area of food fabrication, especially in the area of convenience foods (Samant et al. 1993; Tolstoguzov 1998). Novel food preparation, consists of the direct processing of proteins and other food ingredients into products with defined composition, structure, and function, which to a large extent are controlled by the behavior of protein and polysaccharide components (Tolstoguzov et al. 1985; Tolstoguzov 1998). Also, as a result of research in this field, new methods of protein isolation and protein processing have been developed (Tolstoguzov and Braudo 1983;

Tolstoguzov 1995, 1998). The potential for using proteins and polysaccharides in the preparation of multicomponent gels is the focus of the present research. The enhancement of the functional properties of globular plant proteins in this manner may lead to future manufacturing and processing opportunities.

To optimize the use of protein-polysaccharide systems, for the formation of multicomponent gels, understanding of the effects of environmental conditions on functional properties is essential. The use of model systems, where physiochemical parameters, such as mixture composition, pH and ionic strength can be manipulated, will help assess the nature of polymer interactions and their contribution to rheological properties. This in turn will help assess the ability of a particular composite system to fulfill the role of gelation and provide valuable information when attempting to utilize novel protein-polysaccharide combinations over traditional exclusively protein mixtures (Léger 1992).

The intent of this present research therefore was to determine the optimum parameters leading to the enhanced gelation of globular pea protein when supplemented with various polysaccharides. The impact of these parameters on the gelation of mixed systems will be considered in terms of the interactions or lack of interactions between polymers.

# II. LITERATURE REVIEW

The capacity to form gels under practical conditions is an important aspect of functionality in many food systems. Gels exhibit very diverse microstructural and mechanical properties and as such are very difficult to define. Several review articles have attempted to define gels and characterize gel networks (Ziegler and Foegeding 1990; Clark 1992; Smith 1994). According to Flory (1974), there are three characteristics which constitute a gelatinous state. First, there must be at least two components in the system - a dispersing phase and a dispersed phase. Secondly, both the dispersion solvent and the dispersed component must be equally distributed throughout the gel system. Thirdly, the system must display specific rheological properties typical of a solid. According to Clark (1992), gels can be classified into two types, the *aggregated dispersion* and the *polymer network*. However, it must be noted that the borderline between the two categories of gels is ill defined and gels with intermediate characteristics can emerge.

## A. The Gelation of Globular Proteins

The ability of proteins to form gels is of great importance to the food industry notably within the area of novel food preparation. Network formation is essential to the generation of texture within foods, it provides structural matrices for holding moisture, flavors, sugars and other food ingredients, and it also lends to the stabilization of dispersed phases (Clark 1998). Proteins capable of forming networks can be classified as either fibrous or globular. However the focus of this review will remain on the heat-

setting of globular proteins, which almost exclusively form the first type of food gel, the aggregated dispersion.

**1. Mechanism of Gelation**

Protein gelation can be defined as an ordered aggregation process whereby denatured molecules form a continuous three-dimensional protein network exhibiting structural rigidity. It involves a balance of protein-protein and protein-solvent interactions, and is maintained by a balance of attractive and repulsive forces (Hermansson 1979). Ferry (1948), in a discussion of gelation, proposed the traditional two step process of globular protein gelation. This process involved the complete unfolding of the protein's native structure in order to fully extend its polypeptide chains. The consequential release of the buried domains would then allow for intermolecular interaction necessary to form a three dimensional network.

NATIVE PROTEIN (corpuscular) $\longrightarrow$ DENATURED PROTEIN (long chains) $\longrightarrow$ THREE-DIMENSIONAL NETWORK

Departure from these early views resulted after evidence suggested that interaction of partially unfolded, rather than fully extended, polypeptide chains were responsible for network formation in globular proteins (Clark and Lee-Tuffnell 1986). The presence of soluble aggregates in spherical or bead-like structures, prior to network formation, was later revealed through electron micrographs for a number of proteins. It was discovered that different types of these aggregates existed depending on the overall repulsion charge on the molecule. Consequently, a three-step mechanism known as the

"corpuscular theory", was proposed for protein network formation (Clark and Lee-Tuffnell 1986):

NATIVE PROTEIN (corpuscular) → DENATURED PROTEIN (corpuscular) → SOLUBLE AGGREGATE → GEL NETWORK

**2. Principal Determinants of Globular Protein Gelation**

***a. Denaturation and aggregation.*** Successful thermal gelation of a globular protein is greatly dependent on whether sufficient protein molecules are available to form a continuous matrix. Consequently, unfolding or denaturation of proteins is generally recognized as the initiation step underlying most of the polymerisation processes which occur in gelation (Clark 1998). Denaturation generally involves an alteration in the ordered conformation of polypeptides, of the native protein, to a more unfolded conformation without the rupture of primary covalent bonds (Kinsella 1976). Protein unfolding facilitates aggregation due to the exposure of hidden reactive groups that can then form intermolecular bonds, i.e. hydrogen, hydrophobic, covalent, ionic or electrostatic. Eventually, these aggregates become so large that they are no longer soluble and consequently precipitate. However, when the protein concentration is sufficiently high, a three dimensional gel network can be formed. The unfolding can be affected by any number of structural perturbants (i.e. acid, alkali, urea, or heat). However, the use of heat to denature globular proteins is the most common technique for forming networks within food systems.

The relationship between denaturation and aggregation is an important aspect of protein gelation and will influence the type of network formed. According to Clark and Lee-Tuffnell (1986), the aggregation step must proceed at a slower rate than the unfolding. If the attractive forces between chains are low, denaturation will proceed faster than chain association, and a stable fine gel network that is homogenous and highly elastic can result. However, if denaturation is retarded, protein-protein interactions can proceed too quickly. This leads to gelation prior to sufficient accumulation of free chains, resulting in a course opaque gel composed of larger spherical aggregates which lacks elasticity and is susceptible to destabilization (Cheftel et al. 1985). Several conditions favor denaturation over aggregation. These include a high net charge on protein molecules as occurs at pH extremes, a slower heating rate at the time of denaturation, very low ionic strengths, and finally the presence of chemical denaturants (Léger, 1992). Under these conditions, the activation energy for denaturation is minimized and aggregation is slowed, allowing for orientation of partially unfolded molecules prior to gelation (Clark and Lee-Tuffnell 1986).

***b. Intermolecular forces.*** Several intermolecular forces contribute to the development and maintenance of globular protein gels. The overall contribution of any molecular force is difficult to determine when more than one type of interaction participates in network formation. Their role and degree of involvement is dependent not only on the type of protein but also on the solvent environment in which the protein is dispersed (Cheftel et al. 1985). Ferry (1948), stated that denatured protein gels can be formed only when a critical balance of attractive and repulsive forces is attained. Attractive forces include both noncovalent and covalent interactions.

Noncovalent forces, which contribute to a gel system, include hydrophobic interactions, hydrogen bonding, and electrostatic forces. Since denaturation liberates mainly hydrophobic groups, hydrophobic interactions between newly exposed non-polar groups are believed to be the driving force of globular protein gelation (Cheftel et al. 1985). Hydrophobic interactions have also been suggested as being responsible for the layering or thickening of network strands which lead to improved gel strength and stability and increased opacity (Schmidt 1981). Hydrogen bonding plays a major factor in the increased viscosity preceding the onset of gelation (Schmidt 1981). These bonds contribute to the stabilization of the gel and are enhanced by cooling. Electrostatic forces are induced not only by charged amino acid side chains but are also influenced by the ionic strength of the solvent environment.

Covalent disulfide bonds, formed from exposed sulfhydryl and disulfide groups may also contribute to cross-linking as demonstrated by Ziegler and Foegeding (1990). These covalent bonds contribute to the strengthening of gel structures often rendering them thermally irreversible.

Repulsive forces include electrostatic repulsion and protein-solvent interactions, which tend to prevent intermolecular crosslinking. Conditions favoring repulsion include a high net protein charge, as occurs at pH extremes, very low ionic strength, and the presence of chemical denaturants (Léger 1992; Smith 1994).

*c. **Polymer concentration.*** Another critical parameter in globular protein gelation is the concentration at which proteins will form gel networks. This critical concentration point is highly dependent on the nature of the protein as well as on environmental conditions (Schmidt 1981). Statistically, there is a greater probability of protein-protein

association if the protein concentration is high. Below a minimum concentration, viscosity of the protein solution increases, but a connected gel network never results. It appears that an effective overlapping of functional groups, between adjacent protein molecules or dissociated subunits, is necessary for network formation. Many theoretical models for gelation have included this aspect in its mechanism of gelation (Clark 1992). The Flory-Stockmayer model describes gelation as a sudden event that occurs when the degree of cross-linking between polymers reaches a critical value, called the gel point, at which viscosity then diverges to infinity. Similarly, the "percolation theory" assumes that monomers form small aggregates and at a critical threshold of bonding the gel point is reached, after which the aggregates cross-link throughout the percolation lattice (Ziegler and Foegeding 1990).

Globular protein gels are characterized by their high polymer concentration, greater than that of other polymer network gels, due to the high concentrations of proteins required for network formation (Clark 1998). A critical concentration for gelation does not usually exceed 0.1-0.3% for anionic polysaccharides, while for proteins this value can reach as high as 13%, as seen with 11S broad bean globulin (Tolstoguzov 1995). This difference between gel points, is due to the limited number of possible intermolecular contacts among globular proteins as compared to chain interaction through junction zones seen for polysaccharides (Clark and Lee-Tuffnell 1986). Retention of their corpuscular shape and spatial arrangement during gelation, restricts the number of attachment sites per particle.

### 3. Gelation of Field Pea Protein

Hermansson (1988) classified protein networks broadly into two types: those that aggregate in a random way to produce opaque gels, and those that associate in a more ordered way to give soluble aggregates and transparent gels. The innate physical attributes of transparent gels are appealing to consumers (Bacon et al 1990). Many purified globular proteins will form transparent gels when heated in solution under specific conditions. The expense of generating proteins of the required level of purity, however constrains their use as food ingredients. So far, the only protein used widely by the food industry for the production of clear gel products is gelatin. However, with the high cost of producing animal protein and the increase in people choosing a vegetarian diet, a growing trend towards the development of food protein from plant sources is emerging. In effect, the structure and functionality of numerous plant proteins, particularly from soybean, has been the subject of study for many workers (Catsimpoolas and Meyer 1970; Beveridge et al. 1984; Wang and Damodaran 1991).

The overall scheme of pea protein gelation is illustrated in Figure 1 (Catsimpoolas and Meyer 1970). The protein sol is converted to a high viscosity progel by heat, which then sets to a gel of still higher viscosity upon cooling. The initial heating causes an irreversible dissociation of the globulin polypeptides (Kinsella 1976). Once the sol has been activated to a progel, it can then only be converted into a gel or a metasol. With subsequent heating and cooling, the progel is converted to a gel. The gel can be converted back to a progel by heat and then cooled again to obtain a gel, demonstrating reversibility. The progel or gel, when excessively heated (125°C) forms a metasol which

does not form a gel on cooling. A metasol is also obtained by action of disulfide-cleaving reagents such as mercaptoethanol or sulfite, which causes the chemical modification of functional groups, or by the action of 6M urea.,

The bonds involved in the progel-gel transition seem to be of a noncovalent nature since, based on the protein concentration, a gel can melt at temperatures as low as

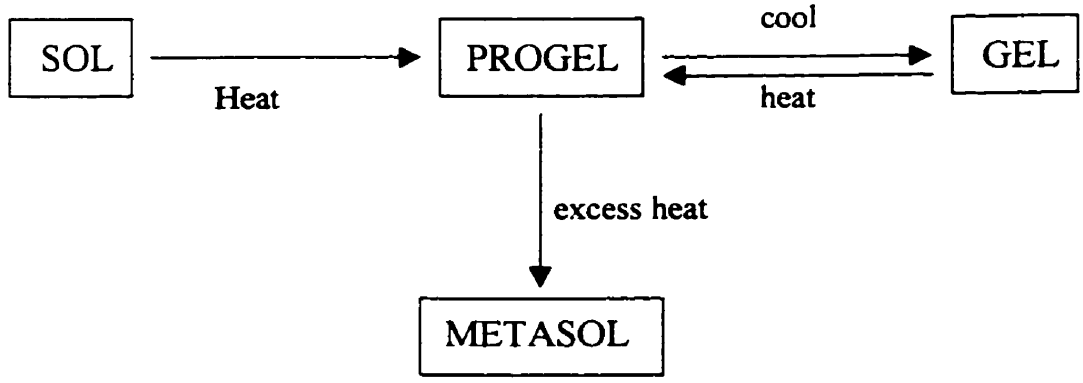



**FIGURE 1. Overall scheme of pea protein gelation (Catsimpoolas and Meyer 1970).**

40°C (Catsimpoolas and Meyer 1970). This however does not exclude the possibility of some limited covalent bonding. The gel, formed by cooling, is rather dependent on the multitude of hydrogen and electrostatic crosslinks, as these two types of bonds are favored by cooling (Beveridge et al. 1984).

## B. The Gelation of Typical Polysaccharides

The second fundamental class of biopolymers, the polysaccharides, are also effective in controlling the rheology of food systems. As in the case with λ-carrageenan and various galactomannans, polysaccharides can be employed as simple viscosifiers to

give shear thinning solutions. In this case, polymer molecules exist as fluctuating disordered chains which contribute to viscosity by virtue of nonspecific entanglement (Dea 1982). Alternatively, polysaccharides can be especially prolific in their capacity to gel the aqueous phase of a food system by forming what is known as a *polymer network* (Clark 1992). Gelation in this case may be instigated by a variety of methods including pH adjustment, ion addition, heating or cooling. Such measures induce disorder-to-order conformations which give rise to specific and permanent interactions between two or more aligned chains, resulting in a rigid framework containing solvent in the interstices (Dea 1982). These associated regions, also known as junction zones, are frequently interrupted by changes in polymer sequence thereby preventing the continuation of an ordered association (Morris 1998). This allows single polysaccharide chains to take part in several regions of ordered conformation involving segments of different chains, consequently giving rise to the three-dimensional gel structure. For polysaccharide gels the relevant interactions are hydrogen bonding, dipole and ionic association. Because all of these interactions involve relatively weak forces, cooperative association of a large number of interactions will be necessary to obtain a firm polysaccharide gel structure (Dea 1982).

The exact mechanism of gelation is dependent on the nature of the junction zones. In the case of gels containing only one polysaccharide, the junction zones in the network can either be ***multiple helices*** or aggregates of ***ordered ribbons*** (Morris 1998). The junction zones of typical polysaccharides are ribbon in nature and follow the gelling process of forming microcrystalline stacks. Examples of polysaccharides that undergo this mechanism are alginate and pectin. Multiple helices refer to another kind of

association, where two or more chains are associated in certain helical directions. This is demonstrated in the case of κ-carrageenan and agarose gelation.

The distribution of polysaccharide chains involved in ordered interactions and disordered chains existing between junction zones is an important factor in determining the final gel properties. In general, the amount of disordered chain conformation present is usually quite low and is conformationally stiffer, making polysaccharide gels comparatively more brittle (Rees 1969). Also, polysaccharide gels are distinguished by their lower polymer concentrations, finer texture and transparency (Clark 1992). Rees (1969) summarized the diverse modes of gelation of different polysaccharides and emphasized the importance of the chemical structure of the macromolecule and the detailed molecular arrangement within the junction zones of the gel. This approach has dominated the study of polysaccharide gels and is central to the currently accepted models of polysaccharide gelation.

## C. Rheological Characterization of Gel Networks

The process of polymer aggregation underlying the gelation of a biopolymer solution can be followed by a variety of techniques (e.g. X-ray scattering and light scattering), but very few of these are capable of directly detecting the development of a full three-dimensional network spanning the system or of measuring the properties of a network as it matures (Clark 1998). Rheology is a science concerned with the deformation and flow of matter. Fundamental rheological methods provide a direct means of mechanically monitoring the transformation of sol to gel and elicits an indirect

means of understanding molecular and structural interactions involved in gel formation (Beveridge et al. 1984). Small amplitude oscillatory rheology is capable of determining the viscoelastic response of gels to dynamic shear stress. In such testing, small deformations are employed thereby preventing sample destruction (Peleg 1987). This allows for multiple measurements to be made and recorded as a function of time or temperature. In fundamental testing, the amplitude and frequency of the imposed deformation are the controlled experimental variables. An additional advantage of fundamental methods is that values obtained from different studies should be comparable regardless of the sample size and the instrumentation used (Léger 1992).

From a rheological point of view, the simplest substances are designated as ideal solids, liquids or plastics that are homogenous and isotropic (Mitchell 1980). However relatively few food systems, including gels, are rheologically simple. All gels possess a solid-like elastic and a fluid-like viscous component simultaneously (Peleg 1987). An elastic component is identified by the direct proportionality between applied stress and the amount of deformation or strain. Energy applied to such a material will be completely restored and there will be no lag between stress and strain as a function of time (Figure 2A). Alternatively, a viscous component shows no elastic recovery and flows in response to an applied stress. Therefore, the applied energy is fully lost and there is a phase angle of 90° between stress and strain over time (Figure 2B). For a viscoelastic material, such as a gel, stress and strain are not in phase, therefore the phase lag can provide an indication of the rheological characteristics and the relative contributions of the elastic and viscous components to the tested material (Figure 2C).

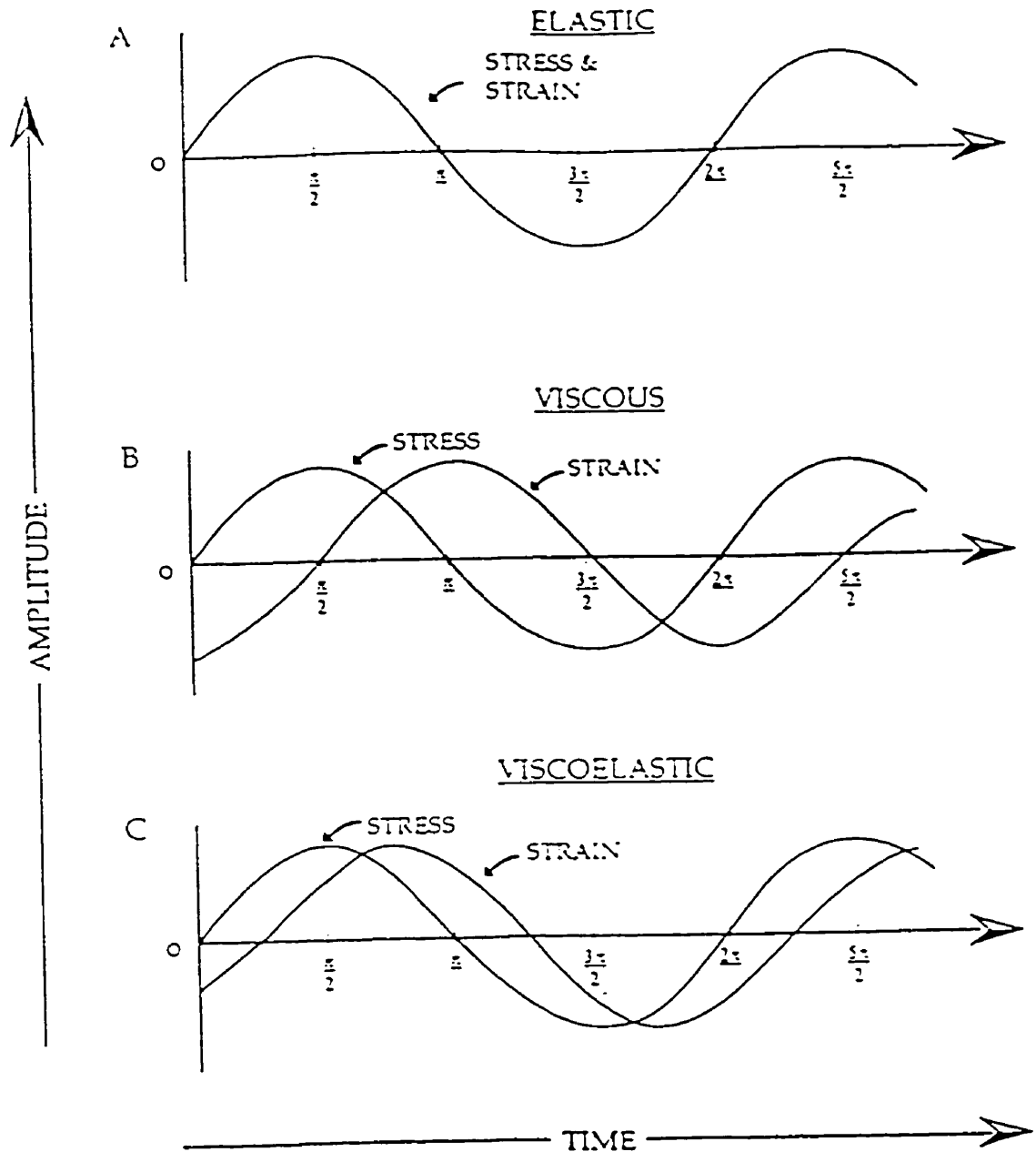



FIGURE 2a-c. **Stresses and strains in dynamic test of elastic, viscous, and viscoelastic materials (Peleg 1987).**

Evaluation of dynamic shear stress response to small amplitude oscillation requires the evaluation of two moduli. The storage modulus (G') is a measure of energy stored due to elastic deformation. The loss modulus (G") reflects a measure of energy lost as heat due to viscous flow within the sample. The loss tangent (tan δ) is the phase angle between stress and strain over time and is a direct measurement of the ratio G"/G'. In principle, as gel networks form, the sample becomes more elastic in nature and G' values will rise while the loss tangent values fall.

## D. Protein-Polysaccharide Interactions

Despite the presence of many potentially reactive groups on both proteins and polysaccharides, conditions within food systems are not usually conducive to their reacting to produce stable covalent linkages (Ledward 1994). Therefore, discussion will focus primarily on the physicochemical nonspecific interactions between these polymers.

Ledward (1994) has outlined that proteins and polysaccharides within a food system can physically interact to give:

1. A liquid two-phase system in which the two polymers are primarily in different phases. This is due to a phenomenon known as *thermodynamic incompatibility.*

2. Homogenous stable solutions can be achieved where the two biopolymer components are either co-soluble or exist as soluble complexes. Electrostatic interaction and hydrogen bonding are involved in the stabilization of such complexes.

3. A two-phase system where both polymer components are largely in the same concentrated phase. This phenomenon of complex coacervation is accredited to the formation of an insoluble electrostatic complex.

Thus, interaction between proteins and polysaccharides may result in three consequences: their cosolubility, incompatibility, or complexing. Co-solubility however, is the least typical situation, in view of the polymeric nature of the macromolecules and the presence of various functional groups (Tolstoguzov 1998). Therefore, focus will be given to complexing and incompatibility. Complexing and incompatibility are seen as mutually connected phenomena being two opposite consequences of non-specific interactions between biopolymers.

**1. Interbiopolymer Complexing**

***a. Complex coacervation.*** When two polymers of opposite net charge are mixed at a low ionic strength, polysaccharides at or above their pK value and proteins below their isoelectric point, the enthalpy of mixing is exothermic and the charge-charge interactions will lead to complex formation (Samant et al. 1993). It has been established that such interactions are primarily electrostatic in nature and will be inhibited at higher ionic concentrations. This energetically favored reaction has been seen most extensively for proteins with acidic polysaccharides (i.e. alginate, pectate) or sulfated polysaccharides such as carrageenan (Tolstoguzov 1991). When interactions between unlike polymer segments are dominant, the two polymers may associate into a liquid or gel-like phase by a process known as complex coacervation. When such a system separates into two

phases, one of them contains higher concentrations of the two reagents termed a coacervate phase. The other, an equilibrium liquid, has a dilute solution of the reagents. Tolstoguzov (1995) demonstrated that at a low ionic strength and a pH below the isoelectric point of gelatin, the interaction of the positively charged macro-ions of gelatin and the negatively charged macro-ions of gum arabic can give rise to insoluble electrostatic complexes, and as a result both components concentrated into a single complex coacervate phase.

***b. Soluble complexes.*** As well as complex coacervation, much attention has been paid to soluble complexes which form due to interactions between macromolecules carrying the same or opposite charge. When solutions of globular protein and sulfated polysaccharide are mixed at a pH value under which the polymers carry opposite charges, an insoluble complex is expected to form. But if for example, the same solution is mixed at a pH value of 9, where complexing is inhibited, and then subsequently brought down below the protein's isoelectric point, the "complex" formed may be soluble (Ledward 1994). Similar results can be obtained by varying the ionic strength of the mixture. Sulfated polysaccharides, with their higher charge density, have demonstrated the ability to form soluble complexes with globular proteins at pH values above the protein's isoelectric point (Tolstoguzov 1991). The affinity between the two biopolymers, resulting in a soluble complex, is believed to be non-electrostatic and dependent on protein structure and conformation (Ledward 1994).

**2. Thermodynamic Incompatibility**

If interaction between polymer segments of like chains is more favorable than between segments of two different chain types, repulsive forces can lead to the mutual

exclusion of each polymer from the domain of the other. At high enough polymer concentrations this can result in separation of the mixture into two distinct phases, known as phase separation. This phenomenon of phase separation has been widely studied (Samant et al. 1993; Ledward 1994; Tolstoguzov 1998). Tolstoguzov and coworkers refer to this phenomenon as thermodynamic incompatibility. Gelatin and gum arabic as seen in the above situation of complexing, can undergo thermodynamic incompatibility at higher ionic strengths and at pH values above the protein's isoelectric point.

The most common consequence of thermodynamic incompatibility is the phenomenon of mutual exclusion. It has been found that the osmotic pressure of a solution of incompatible polymers exceeds the sums of individual osmotic pressures (Tolstoguzov 1995). This is interpreted as an exclusion of each polymer from the domain of the other by mutual repulsion, which in turn increases the effective concentration of each. Exclusion effects are strongly dependent on concentration, becoming undetectable in very dilute polymer solutions, where the individual chains are widely separated. Excluded volume effects are also dependent on flexibility, shape, and size of macromolecules (Semenova et al. 1991). Since the number of energetically unfavorable contacts that a chain can make increases with increasing flexibility and size, conformationally mobile polymers are more effective in excluding other polymers than are more constrained ones (Ledward 1994).

## E. Gelation of Protein-Polysaccharide Mixtures

Composite gels are produced from a mixture of two or more gelling agents, or a single gellant and nongelling components. There are a variety of ways in which these

components can interact and this will affect the properties of a multicomponent gel. Influencing factors include: thermodynamic incompatibility of the components, their mutual reactivity or potential for interaction, and for combinations of two or more gellants, their respective mechanisms of gelation.

**1.Types of Multicomponent Gels**

***a. Filled gels*** Filled gels are obtained if one component forms a continuous network over the entire system while other polymeric components, known as gel fillers, are interspersed throughout the primary network (Tolstoguzov 1998). Two types of filled gels can be distinguished depending on the phase state of the system (Aguilera 1992; Ziegler and Foegeding 1990). The "single polymer network" or "filled gel type I" is the simplest microstructure and is formed in a single phase system where only one polymer gels and the filler remains soluble. In a two-phase system, a "composite network" or "filled gel type II" is formed where the dispersed phase consists of particles of liquid or gel. In this case, thermodynamic incompatibility causes phase separation resulting in both polymers forming separate segregated networks. An example of a type II filled gel is that produced by the addition of wheat or potato starch to red hake surimi (Aguilera 1992).

***b. Mixed gels*** Mixed gels can be treated as a particular case of homogenous "interpenetrating polymeric networks". In this case, both polymers gel and form relatively independent networks that interlace one another and are continuous throughout the sample (Ziegler and Foegeding 1990). Mixed gels are formed when the concentrations of biopolymers in a solution exceed the critical concentration for gelation.

Interesting properties are exhibited by mixed gels, where one component forms a thermosetting gel and the other a gel which sets by the action of ions. An example of such a gel is that of pectinate and denatured ovalbumin (Tolstoguzov 1995). The ionic gel network has been shown to control the formation of the thermosetting gel.

*c. Complex gels.* Complex gels are formed when interactions among the components lead to their physical association. A "nongelling" component may associate with the primary network in a random fashion via nonspecific interactions. Such interactions may reduce the flexibility of the primary network chains and add to the rigidity of the complex gel (Tolstoguzov 1998). Examples of such gels are those formed by gelatin-sodium alginate complexes and gelatin-low ester pectin complexes. It is worth noting that neither polymer formed gels alone under the conditions used for gelation of the complex. This phenomenon is attributed to the occurrence of salt linkages between proteins and anionic polysaccharides. The conditions required to produce gels from protein/polysaccharide complexes and their resulting properties have been discussed by Stainsby (1980).

*d. Copolymer gel.* Another unique type of multicomponent gel is where two or more polymers may copolymerize to form a single heterogeneous network. Bovine serum albumin-ovalbumin gels have been demonstrated to be of this type (Ziegler and Foegeding 1990).

## 2. The Effect of Thermodynamic Incompatibility on Gelation

The incompatibility of biopolymers can give rise to several different effects during the multistage gelation process. Incompatible polymers mutually concentrate each

other consequently lowering the gel point concentration of each component. Since the shear modulus of a gel is usually proportional to the square of its concentration, small additions of a polysaccharide can increase the elastic modulus of a gel several times (Ledward 1994). Tolstoguzov (1995) demonstrated this effect through the addition of a very small amount of dextran to gelatin solutions which increased the elastic modulus of the gel several fold.

Another possible effect of incompatibility is the intensification of aggregation of denatured molecules. This is due to the unfolded conformation, the increased exposure of hydrophobic groups and the lowered hydrophillicity of denatured protein molecules. All these factors favor the aggregation of denatured molecules thereby inhibiting renaturation and increasing both the elastic modulus and the permeability of the multicomponent gel (Samant et al 1993). By increasing the protein's thermodynamic activity under incompatible conditions, Tolstoguzov (1991) demonstrated this effect on broad bean legumin-polysaccharide gelation.

# MATERIALS AND METHODS

## A. Source of Materials

Yellow field pea flour (*Pisum sativum*), which was air classified to give protein-rich fractions, was obtained from Parrheim Foods (Saskatoon, SK.). Parrheim Foods (Saskatoon, SK) utilizes dry processing in order to isolate protein fractions. The protein-rich fraction was stored in its original packaging at room temperature.

Food grade locust bean gum and guar gum were obtained from Zumbro Inc (Hayfield, Minn.). These neutral galactomannans consist of a mannan backbone with partial substitution at mannose C-6 sites by α-D-galactose. Locust bean gum and guar gum differ primarily in their galactose to mannose ratio which is of the order of 1:2 for guar gum and 1:4 for locust bean gum (Gidley et al. 1991). Rheological parameters (G' and tan δ) for 1% aqueous solutions of these non-gelling polysaccharides were: 123 Pa and 0.143 and 145 Pa and 0.160, respectively. κ-Carrageenan (food grade) was obtained from FMC Corporation (Philadelphia, Penn.). The carrageenan backbone consists of a linear alternating sequence of 1,3-linked β-D-galactose and 1,4-linked α-D-galactose units. It is a sulfated galactan and the κ-form contains one sulfate group every disaccharide repeating unit (Williams and Langdon 1996). The G' and tan δ values of a 1% aqueous solution were 344 and 0.122, respectively. All polysaccharides were used without additional purification. The rheology measurement of pure polysaccharide systems were based on the same conditions set for protein-polysaccharide systems (see section D).

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

## B. Preparation of Protein Isolate

The preparation of plant protein isolates has often been accomplished through isoelectric point precipitation followed by extraction using alkaline or acidic conditions. However, evidence has suggested that such conditions can lead to severe protein denaturation (Arntfield and Murray 1981). Changes in protein conformation, during extraction, may significantly affect protein unfolding during heating and the resulting network formation. Therefore, milder isolation procedures, such as the micellization technique of Murray and coworkers (1978), have been employed in the present study to ensure that native proteins are not altered.

Figure 3 details the steps used to isolate field pea protein in the present study. The pea flour was mixed with 0.3 M NaCl at a ratio of 1:10 and stirred constantly for 30 min at 37°C. The protein slurry was centrifuged at 10 000 x g for 15 min in a Sorval General Purpose RC-3 Automatic Refrigerated Centrifuge equipped with a HG-4L rotor head (Newton, CT). The supernatant was then separated and dispersed in cold water, at a ratio of 1:3 and left to stand at room temperature for 3 hr. The protein micellar mass (PMM) was collected by decanting the supernatant. The residual protein mass was collected and dialyzed against distilled water at 4°C for 72 hours to remove excess salt. The dialyzed protein fraction was then frozen at –32°C and freeze-dried at approximately -55°C for at least 48 hr at 190 mtorr in a Virtus freeze-dryer (Gardenir, NY). The dried PMM samples were stored in airtight containers at room temperature until required for use.

Protein content of the field pea isolate was 86% (N x 5.70) as determined by the micro-Kjeldahl method (A.O.A.C. 1975). The moisture and ash content of the lyophilized PMM were determined (A.O.A.C., 1975) to be 4.1% and 2.0%, respectively.

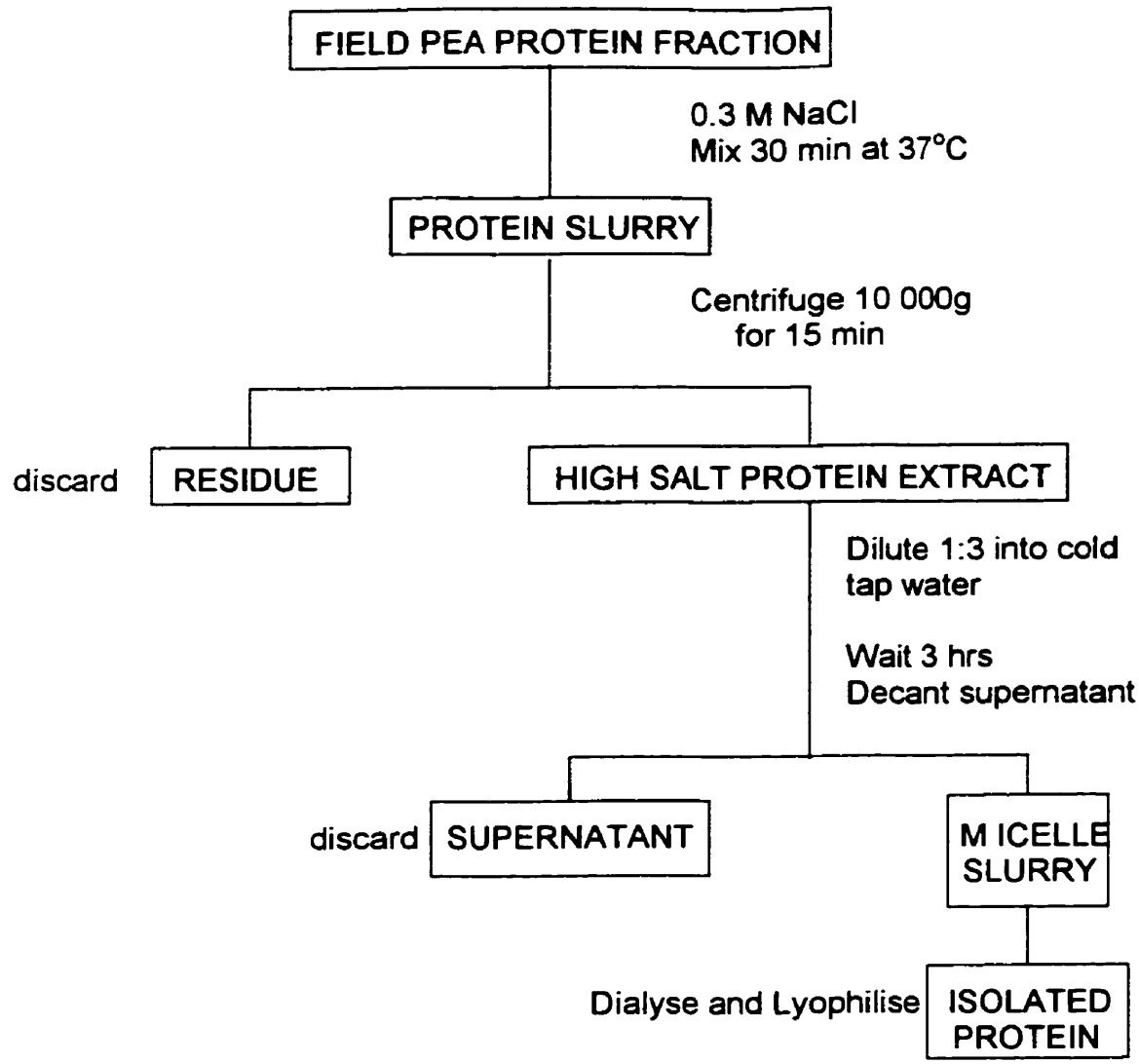



FIGURE 3. The isolation of protein from yellow field pea flour using the procedure of Murray et al. (1978)

Danielsson (1949) determined the isoelectric points of pea legumin and vicilin as pH 4.8 and pH 5.5 respectively. The G' and tan δ values of a 20% protein gel at its natural pH were 956 Pa and 0.140, respectively. It has been reported that for pea protein the minimum concentration for gelation is approximately 8.0% (Bacon et al, 1990).

### C. Sample Preparations

Suspensions with varying pea protein concentrations, protein-polysaccharide ratios, protein-salt ratios and pH were made. The specific levels for each of these elements varied with the different experimental designs used during the course of the study. These are described later under the section on *Statistical Design and Analysis.* Lyophilized forms of both protein and polysaccharide were mixed at desired amounts in a 15 mL test tube. This dry mixture was then hydrated with a specific NaCl solution, adjusted with respect to pH and mixed for at least 60 seconds using a vortex. Samples were then made up to a volume of 10 mL. The pH of the samples were adjusted by dropwise addition of a small amount of NaOH (0.5M) or HCl (0.5M). Each mixture to be tested was allowed to stand for at least 30 min to allow for stabilization of the mixture and to allow the settling of air bubbles. After the elapsed time, the pH of the mixtures were rechecked to ensure stability had been reached.

### D. Rheology

All rheological measurements were made using a Bohlin VOR rheometer (Bohlin Reologi, Inc., Edison, NJ), operated in the small-amplitude oscillatory mode. The rheometer was equipped with 30-mm parallel plate geometry and with a torque bar

calibrated to 93.1 g-cm. Input strain amplitude for dynamic analysis was 0.02 and sensitivity was set at 10x.

Approximately 1 mL of sample was transferred to the lower plate of the parallel-plate geometry at room temperature. This volume was adequate to fully cover the lower plate and obtain a gap width of 1.00 mm when the upper plate was lowered. A 15-cm strip of masking tape was wrapped around the circumference of the cylinder supporting the lower platen to form a well. Mineral oil was applied to the constructed well to prevent sample drying during heating. 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. Rheological data was collected, during the frequency sweep, every 2.0 min with a thermal equilibrium time of 10s. Frequency sweeps of the final product were conducted over a range of 0.01-10 Hz at 25°C.

Rheological properties were expressed in terms of storage modulus (G') and loss modulus (G"). The loss tangent could then be calculated as $\tan \delta = G''/G'$. The G' and $\tan \delta$ values at 1 Hz of the final frequency sweep were taken for comparison of the rheological results of the gelled products. As frequency increases, the slope of G' and G" values remain parallel to each other (Appendix A). Since different frequencies do not have a particular influence on mechanical spectra, the selection of one particular frequency, in which to look at rheological parameters is acceptable.

### E. Differential Scanning Calorimetry

Differential Scanning Calorimetry (DSC) was used in this study to monitor the conformational stability of protein-polysaccharide mixtures exposed to various

physicochemical influences. Measurements were carried out using a Dupont 9900 Thermal Analyzer with a Dupont 910 Cell Base (Dupont Instruments, Wilmington, DE). Aliquots of 20 μL of the sample mixture were placed in tared aluminum DSC pans (Part Nos. 900790.901 and 900796.901, Dupont Instruments). The pans were then hermetically sealed and reweighed to obtain an accurate weight of the sample. Each sample, along with an empty reference plan, was mounted with silicone heat sink compound in a standard DSC cell (cell calibration coefficient =1.028; onset slope = -16.35). The cell was heated over a temperature range of 30-115°C at a rate of 10°C/min. Each sample mixture was analyzed in duplicate.

Dupont's DSC General Analysis Utility program, Version 2.2 was used to analyze the thermal properties of the sample mixtures. A temperature corresponding to the maximum heat flow was taken as the denaturation point (Td) and the denaturation enthalpy (ΔH) was calculated by numerical integration of the peak area. Values obtained from DSC were examined in duplicate and averaged.

### F. Solubility Assessment

Solubility behavior provides an excellent index of protein functionality and aids in monitoring the effects of various modifications (i.e. addition of polysaccharide) on its functional potential. The solubility of the mixed solutions, prior to gelation, were determined spectrophotometrically utilizing the Coomassie protein determination method (Pierce Chemical Co. 1995). Prior to testing, the mixed solutions were clarified via centrifugation (10 000x g for 5 min). Solubility measurements were recorded as grams of protein/ 100 g and were examined in duplicate and averaged.

## G. Statistical Design and Analysis

Both factorial designs and response surface methodology (RSM) were utilized for the experiments in the present study. Experimental designs were constructed and data analyzed using an IBM personal computer and Design Expert V5.31 software package.

RSM encompasses a collection of statistical and mathematical techniques useful for developing, improving and optimizing processes and formulations of new products (Myers and Montgomery 1995). The nature of the mathematical model in RSM is a predictive equation, predicting the value of the response variable for given levels of the factors. This implies that all factors are fixed and quantitative. Most applications of RSM are sequential in nature. Often at the outset of a response surface study there is an extensive list of factors that could be significant in explaining the response. This necessitates a *screening experiment*, designed to investigate these factors with a view towards eliminating the unimportant ones. Myers and Montgomery (1995) refer to this screening experiment as *phase zero* of a response surface study. Once the significant variables are identified, *phase one* is carried out to determine if the current levels or settings of the independent variables result in a response value near the optimum. *Phase two* of the response surface study begins when the process is near the optimum. At this point a model is desired that will accurately approximate the true response function within a relatively small region around the optimum. Once an appropriate approximating model has been obtained, this model may be analyzed to determine the optimum conditions for the process. On this basis, the RSM applied in the present study consisted of three separate experiments: Phase Zero - Screening Experiment, Phase One - Preliminary Optimization and Phase Two - Confirmational Optimization.

**Phase Zero - Screening Experiment.** Two-level fractional factorial experiments were carried out separately for all three polysaccharides, to examine main and interaction treatment effects on the responses: storage modulus (G'), loss tangent (tan δ), denaturation temperature (Td), enthalpy of denaturation (ΔH) and solubility. The factors involved were protein concentration (10% and 20%), protein to polysaccharide ratio (20 and 100), protein to NaCl ratio (10 and 30) and pH (4 and 9). The single block design consisted of no replication and encompassed four center points.

**Phase One - Preliminary Optimization.** Based on the screening experiment, a second series of experiments were conducted following a central composite RSM design. This design contained the elements of a two level fractional factorial design plus center and axial points. As a result, several different models could be applied to describe the data. The independent factors included protein concentration (15 and 25%), protein:polysaccharide ratio (15 and 75) and pH (6 and 8). Level of NaCl not considered after phase zero results were examined, and was consequently kept constant at protein:salt ratio of 1:20. Center and axial points were determined based on an alpha value of 1.6.

**Phase Two - Confirmational Optimization.** A final set of experiments were subsequently carried out in attempts to corroborate and substantiate the results obtained in phase one. A central composite RSM design, as seen in phase one, was utilized differing only in an alpha value of 1.0. The independent factors included protein concentration (15 and 25%), protein:polysaccharide ratio (15 and 75) and pH (6 and 8). The difference in alpha value established different axial points as compared to phase 1. The phase two design eliminated meaningless axial points (i.e. negative values), focussed

in on areas where significant findings were being observed, and allowed for a fuller representation of previous findings by the addition of previously untested factorial points.

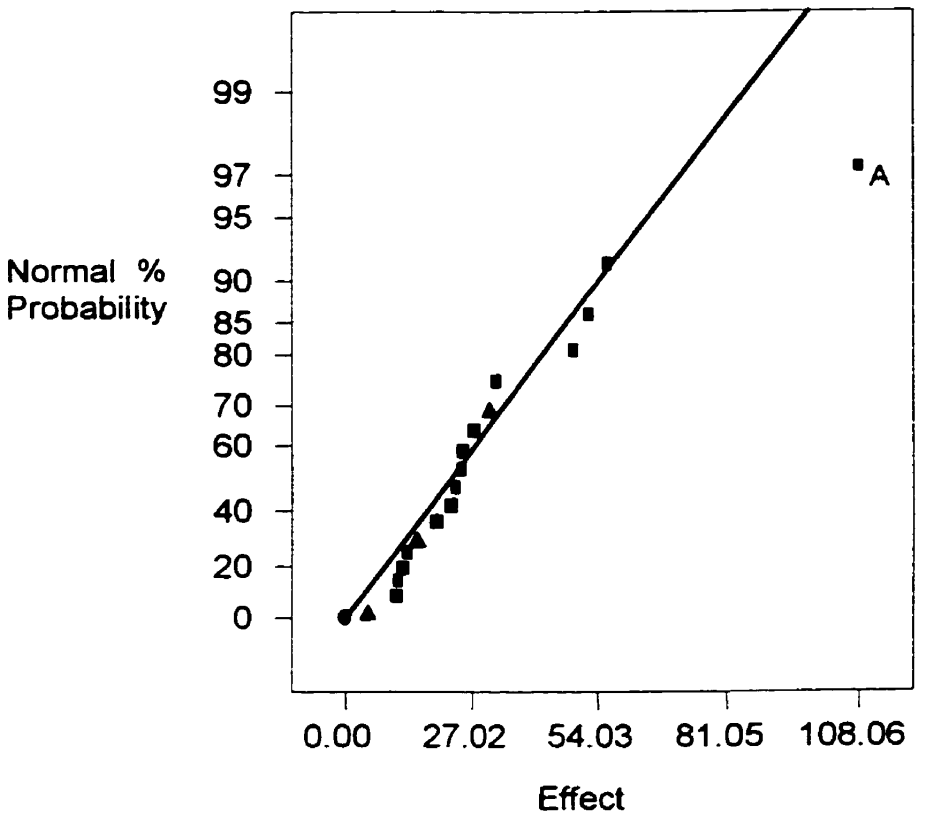
**Statistical Analysis.** Data from physical measurements were analyzed by analysis of variance to determine significant effects on responses. Statistical evaluation of DSC and solubility results, in response surface experiments, were performed on the combination of phase one and two data. Model fitting was performed on the combined experiments of phase one and two, utilizing numerical and graphical optimization, to determine optimum physiochemical conditions for maximizing G' and minimizing tan δ.

# IV. RESULTS

## A. LOCUST BEAN GUM SYSTEMS

### 1. Phase Zero - Screening Experiment

The rheological data (G' and tan δ) obtained with mixed locust bean gum and field pea are shown in Appendix 1. Data were generated based on a 2-level fractional factorial design with protein concentration (A), protein-polysaccharide ratio (B), protein-salt ratio (C) and pH (D) as variables. Given the normal probability plot (Figure 4) and analysis of variance (Appendix 2) of the effect estimates, the only significant effect on G' values was that of protein isolate concentration ($p < 0.005$). The positive relationship observed between protein concentration and G' can be explained by the increased probability of crosslinking due to increased polymer concentration (Clark and Lee-Tuffnell 1986). Despite the increase in G', networks formed still exhibited very low and variable experimental values making evaluation difficult and unreliable. Based on analysis of variance data (Appendix 3), tan δ values were not shown to be dependent on any of the tested variables. Locust bean gum, like guar gum, is characterized as a neutral, non-gelling polysaccharide. As a neutral polymer, locust bean gum has the potential to promote thermodynamic incompatibility within a mixed biopolymer system (Tolstoguzov 1992). It has been shown that the mechanism of improved gelation via an incompatible system is through mutual exclusion of the protein. Given that the addition of locust bean gum into a pea protein system resulted in no increased gelation, as will be seen for guar gum later, it is assumed that the exclusion phenomenon did not occur. A reason for this may be due to the polysaccharide's structure. Compared to guar gum, locust bean gum




Factors: A: Protein Concentration B: Protein-Polysac. Ratio
C: Protein-NaCl Ratio D: pH

FIGURE. 4. Normal probability plot showing significant factors with respect to the storage modulus G' of pea protein-locust bean gum systems at 1 Hz. *(Screening Experiment)*

has a lower degree of galactose substitution along its mannose backbone consequently leaving many "smooth" or unsubstituted regions (Goycoolea et al 1995). It is possible that some limited form of association or entanglement may have occurred between polypeptides and these regions, consequently inhibiting incompatibility. This type of association has been seen with locust bean gum and carrageenan (Williams and Langdon 1995). A study conducted by Murayama et al (1995) on mixed gels of κ-Carrageenan and galactomannans, showed that κ-carrageenan exhibited a synergistic interaction when combined with locust bean gum but not when combined with guar gum. The difference in galactose substitution ratios between locust bean gum and guar gum was considered responsible for the differing rheological properties of the mixed gels.

DSC data showed that the thermal denaturation temperatures (Td) of the mixed systems were significantly effected by protein concentration and pH (Appendix 4). Higher protein levels contribute to a shielding effect against thermal degradation lending to increased temperatures at which denaturation takes place (Table 1). A positive relationship between Td and pH was also seen. At pH 4, the overall net repulsive charge on the pea protein was low when considering that the isoelectric point of pea protein is between 4.8 and 5.5 (Danielsson 1949). Since the protein was close to its isoelectric point, the chance of precipitation became more imminent and the overall stability of the mixture was diminished. This allowed the protein to unfold more easily and at a lower temperature. As the pH moved upwards towards 9, denaturation was delayed since protein-solvent interactions increased allowing for better mixture solubility and stability.

Solubility determination, prior to gelation, showed that protein concentration, pH, the interaction between protein concentration and pH, and salt had a significant effect on

TABLE 1. Effect of protein concentration and pH on the thermal denaturation temperature Td (°C) of mixed pea protein-locust bean gum systems[a].

| Protein Concentration (%) | pH 4 | pH 9 |
|---|---|---|
| 10 | 69.50 | 92.67 |
| 20 | 79.95 | 99.97 |

[a] Values displayed in table are based on a range of protein-polysaccharide and protein-NaCl ratios. Since these factors showed no statistical significance to protein solubility, values were consequently averaged

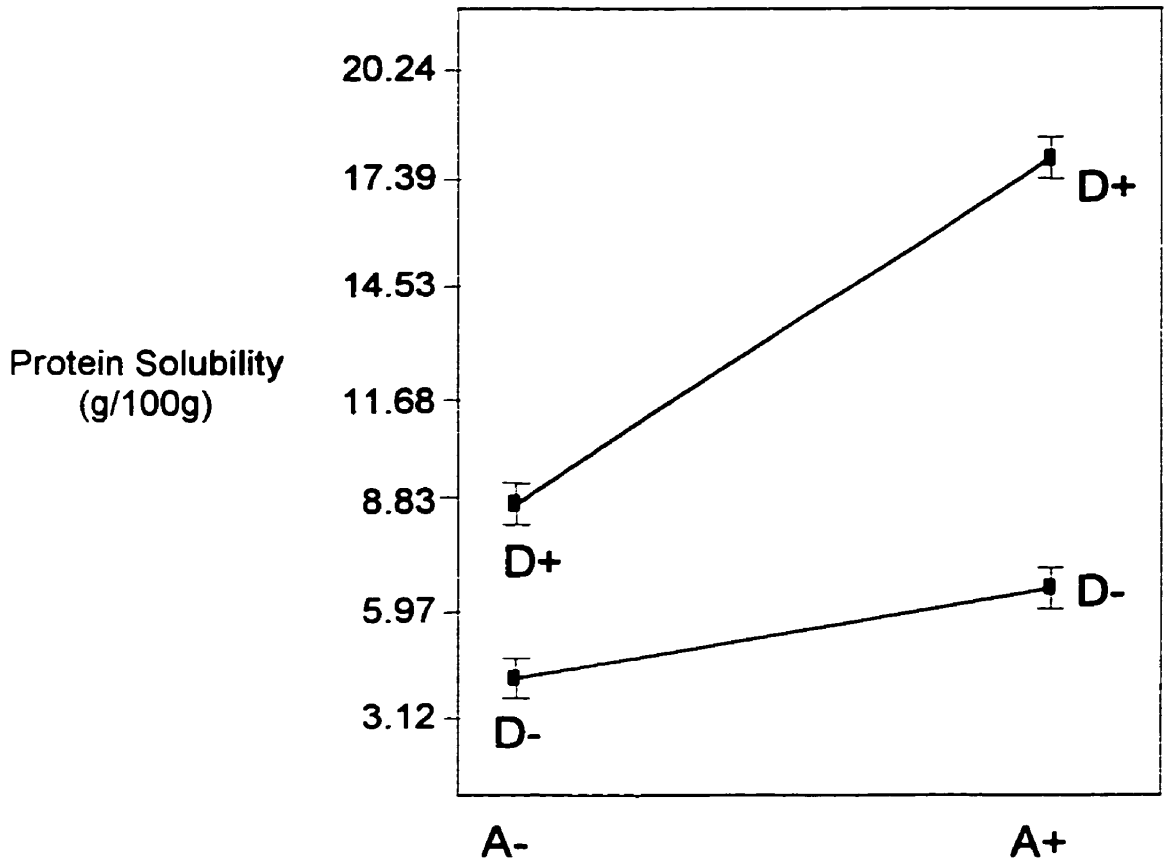
solubility ($p < 0.0001$) (Appendix 5). The direct effect of pH on solubility is attributable to the fact that as pH is moved away from the isoelectric point (IEP), protein-solvent reactions are favored over protein-protein reactions (Table 2). The low solubility seen at pH 4 is due to the low net repulsive charge on the protein lending to increased protein-protein interactions and subsequent aggregation. This was depicted by the homogenous milky-white appearance of protein solutions in this acidic environment. As seen in Figure 5, protein concentration had more effect on solubility when at an alkaline pH. This supports the fact that at higher pH levels, the solubility of pea protein is favored so that the effect of protein concentration is more noticeable. At pH 4, the protein system was unstable resulting in minimal solubility regardless of the protein level examined. The positive effect of salt on solubility, as seen in Table 2, is a common observation attributable to the "salting in" effect where salts react with oppositely charged groups on the protein consequently decreasing protein-protein interactions (Kinsella 1976).

Since there was no notable contribution by locust bean gum on the rheological properties of pea protein systems, further investigations with this polysaccharide were unwarranted. This does not imply that locust bean gum is entirely unable to improve protein gelation. Rather, this gum was not seen to be beneficial under the conditions of this study. The lack of impact made by this gum on protein solubility is similar to that which will be reported for guar gum. This outcome may be characteristic of mutual exclusion and may indicate that some form of incompatibility is present in the composite system. However, the fact that polysaccharide levels did not influence the Td values, showed that locust bean gum did not encourage the incompatibility or interaction between polymers to a sufficient extent to affect conformation or rheology.

TABLE 2. Effect of protein concentration, pH and protein/NaCl ratio on the protein solubility (g /100g) of mixed pea protein-locust bean gum systems[a].

| Protein Concentration (%) | Protein/NaCl Ratio | pH 4 | pH 9 |
|---|---|---|---|
| 10 | 10 | 4.9 | 9.4 |
| 20 | | 7.2 | 19.7 |
| 10 | 30 | 3.6 | 8.2 |
| 20 | | 6.1 | 16.2 |

[a] Values displayed in table are based on a range of protein-polysaccharide ratios. Since protein- polysaccharide ratio showed no statistical significance to protein solubility, values were consequently averaged




Factors: A: Protein Concentration
" - " indicates 10% protein
" +" indicates 20% protein

D: pH
" - " indicates pH 4
" + " indicates pH 9

FIGURE 5. Interaction effect of protein concentration and pH on the protein solubility (g/100g) of pea protein isolate-locust bean gum systems. *(Screening Experiment)*

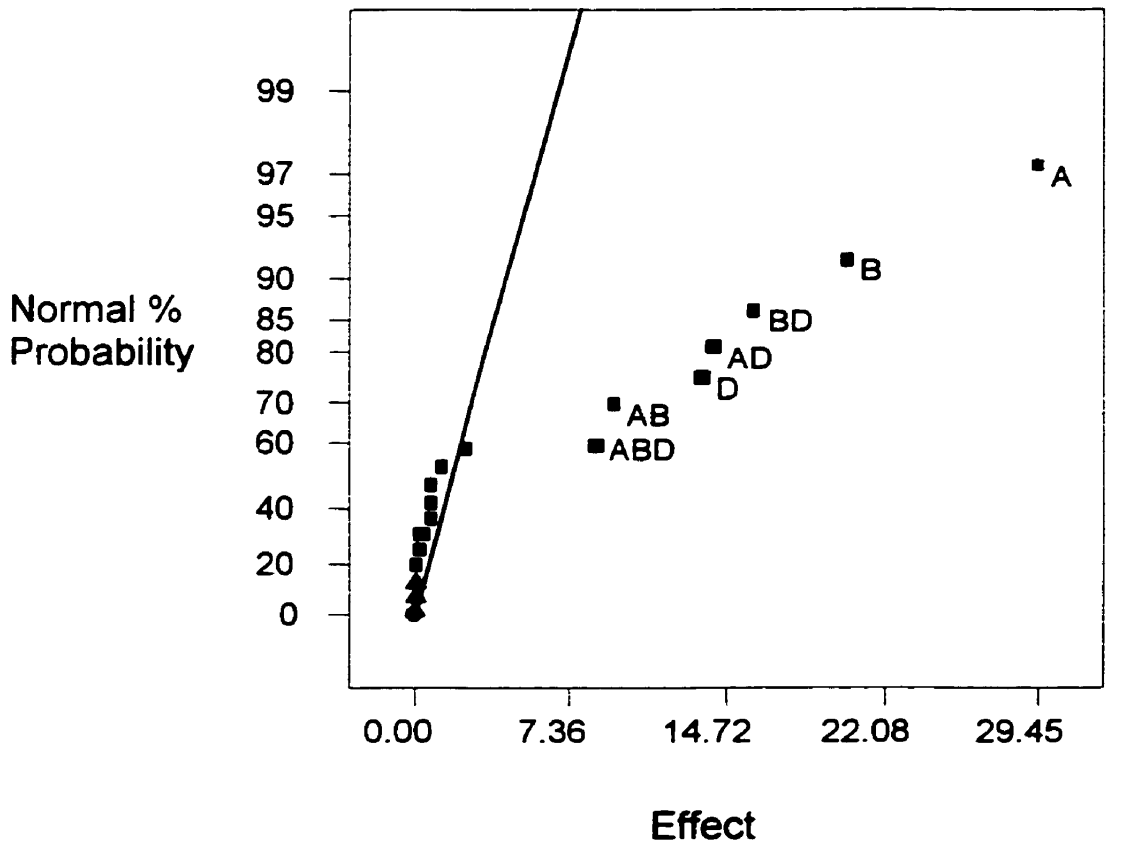
## B. GUAR GUM SYSTEMS

### 1. Rheology

#### a. Phase Zero - Screening Experiment

The rheological data for mixed pea protein-guar gum systems is given in Appendix 6. Two levels of each factor were set: 10 and 20% for protein concentration, 20 and 100 for protein-polysaccharide ratio, 10 and 30 for protein-salt ratio and pH levels at 4 and 9. Since the range of G' responses was large, a manipulation in the response, via square root transformation, allowed for a better fit of the model. The normal probability plot for G' is shown in Figure 6. All of the effects that lie along the line are negligible to the response, whereas the large effects are seen by variables far from the line. The analysis of variance is summarized in Appendix 7. Based on the normal probability plot and the p-values generated, it was shown that protein concentration, protein-polysaccharide ratio and pH all had highly significant effects on G' values for the mixed gels ($p < 0.0001$). In addition, two-way and three-way interactions (i.e. ABD) between these three variables were also statistically significant ($p < 0.0001$).

In terms of polymer levels, an increase in protein concentration resulted in an increase in G' while the relationship between G' and protein-polysaccharide ratio displayed a negative relationship (Table 3). Note that a decrease in protein-polysaccharide ratio corresponds to an increase in polysaccharide concentration within the mixed system. Pea protein concentrations of 15 and 20% always formed networks, while at low concentrations (i.e. 10%) the mixture usually remained in a liquid-like state after the




Factors: A: Protein Concentration B: Protein-Polysac. Ratio
C: Protein-NaCl Ratio D: pH

FIGURE 6. Normal probability plot showing significant factors with respects to the storage modulus G' of pea protein-guar gum systems at 1 Hz. Note: square root transformation was applied) *(Screening Experiment)*

TABLE 3. Effect of protein concentration, protein-polysaccharide ratio, and pH on the storage modulus G' (Pa) of mixed pea protein-guar gum systems[a].

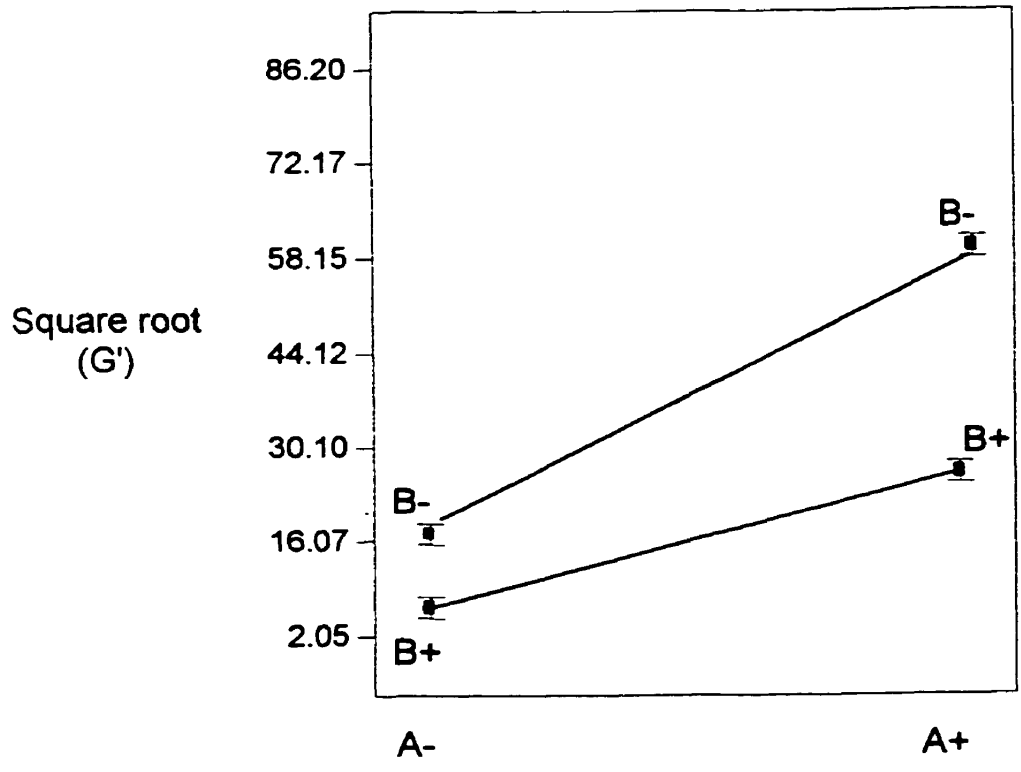
| Protein Concentration (%) | Protein-Polysac. Ratio[b] | pH 4 | pH 9 |
|---|---|---|---|
| 10 | 20 | 197.0 | 402.0 |
| 20 | | 861.6 | 6834.6 |
| 10 | 100 | 94.9 | 7.3 |
| 20 | | 625.1 | 744.1 |

[a] Values displayed in table are based on a range of protein/NaCl ratios. Since protein/NaCl ratio showed no statistical significance to G', values were consequently averaged

[b] Decrease in protein-polysaccharide ratio corresponds to an increase in polysaccharide concentration within the mixed system

cooling-heating phase was completed. For example, a mixture at pH 9 with 10% protein, a protein-polysaccharide ratio of 100, and a protein-salt ratio of 10 generated a system with a G' of only 11.2 Pa. In these cases, it was presumed that the critical point of gelation had not been met. Therefore an inadequate amount of protein was present to allow for the formation of a three-dimensional network. This observation has been made by other researchers for other globular proteins using small amplitude oscillatory rheology, and relates to the fact that the probability of crosslinking among macromolecules is greater at higher polymer concentrations (Léger and Arntfield 1993). Burova et al (1992) found that the gelation threshold of broad beans at pH 4.2 and 7.0 were estimated to be 11% and 13% respectively. The relatively high gelation threshold value of this protein was explained by the fact that significant fractions of the polypeptide chain did not participate in the formation of a three-dimensional network. A similar explanation may be applied to the pea protein isolate in this study. The significant interaction effect (AB) indicates that increases in G' as a result of increasing protein concentration was dependent on the protein-polysaccharide ratio. Figure 7 illustrates how protein concentration had an impact on G' only when protein-polysaccharide ratios were low. Even at protein concentrations below the presumed critical point, thin gels were formed due to the increased level of polysaccharide. In contrast to the mixture previously seen with a G' of 11.2 Pa, the same system with a protein-polysaccharide ratio of 20, resulted in a G' of 443 Pa. While the mechanism still remains unknown, it is evident that the presence of polysaccharide aided in protein network formation.

As shown by the analysis of variance (Appendix 8), protein isolate and polysaccharide




Factors: A: Protein Concentration
" - " indicates 10% protein
" +" indicates 20% protein

B: Protein-Polysac. Ratio
" - " indicates ratio of 20
" + " indicates ratio of 100

FIGURE 7. Interaction effect of protein concentration and protein-polysaccharide ratio on the storage modulus G' (Pa) of pea protein-guar gum systems (*Screening Experiment)*. Note: square root transformation was applied

levels played a dominant role in determining the tan δ response, as did their interaction ($p < 0.05$). High concentrations of pea protein in the mixture resulted in lower tan δ values, corresponding to improved gel networks. Similarly, desirable tan δ values were consistently generated at low protein-polysaccharide ratios (Table 4). The relationship between these two observations was further seen in the interaction graph (Figure 8), which indicated that protein concentration had no effect on tan δ when polysaccharide levels were high. It is clear that the presence of guar gum has a positive effect on gelling parameters. The likelihood of guar gum alone forming these composite networks is highly improbable since this polysaccharide is characterized as having no gelling capacity (Murayama and Kawabata 1995). A 1% aqueous solution of guar gum (similar to a protein-polysaccharide ratio of 20 at a protein concentration of 20%) gave G' and tan δ values of only 145 Pa and 0.160 respectively. Therefore in this case it is presumed that pea protein is the main contributor to network formation. These observations support the assumption that the presence of guar gum promotes incompatibility within the protein system. Since the presence of guar gum would facilitate the exclusion of protein, it is the effective concentration of protein which would enable network formation, rather than the concentration of protein initially added to the system.

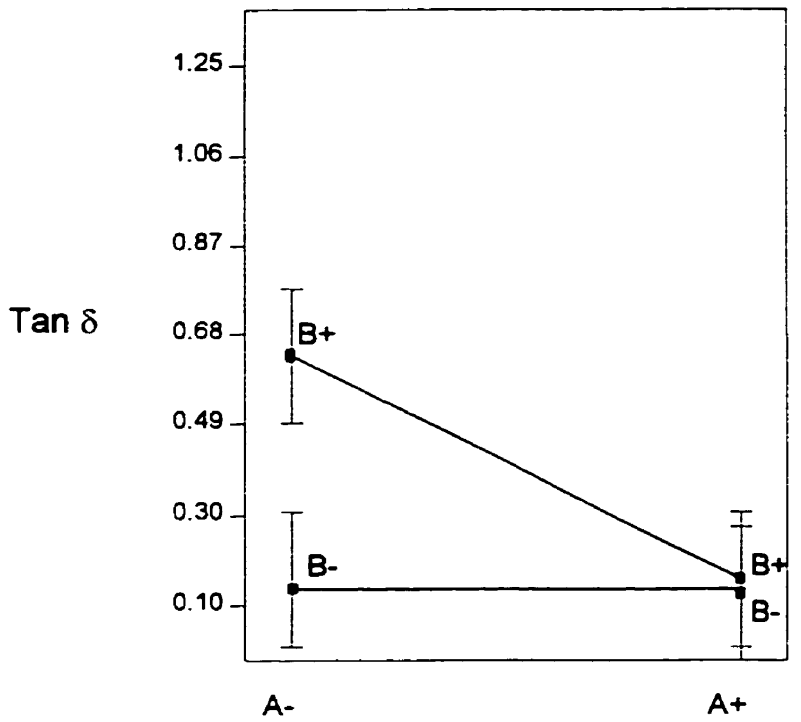
The pH level had a significant positive effect on G' values ($p < 0.0001$) and therefore on the strength of the network formed (Table 3). This was further compounded by the interactions seen between pH and polymer concentrations. Figures 9 and 10 illustrate respectively, how protein and polysaccharide levels made greater contributions to G' when at a high pH. These trends may be related to the solubility of the system at these different pH levels.

TABLE 4. Effect of protein concentration and protein-polysaccharide ratio on the loss modulus (tan δ) of mixed pea protein-guar gum systems[a].

| Protein Concentration (%) | Protein-Polysaccharide Ratio[b] 20 | 100 |
|---|---|---|
| 10 | 0.159 | 0.580 |
| 20 | 0.126 | 0.158 |

[a] Values displayed in table are based on a range of protein-NaCl ratios and pH levels. Since these factors showed no statistical significance to protein solubility values were consequently averaged

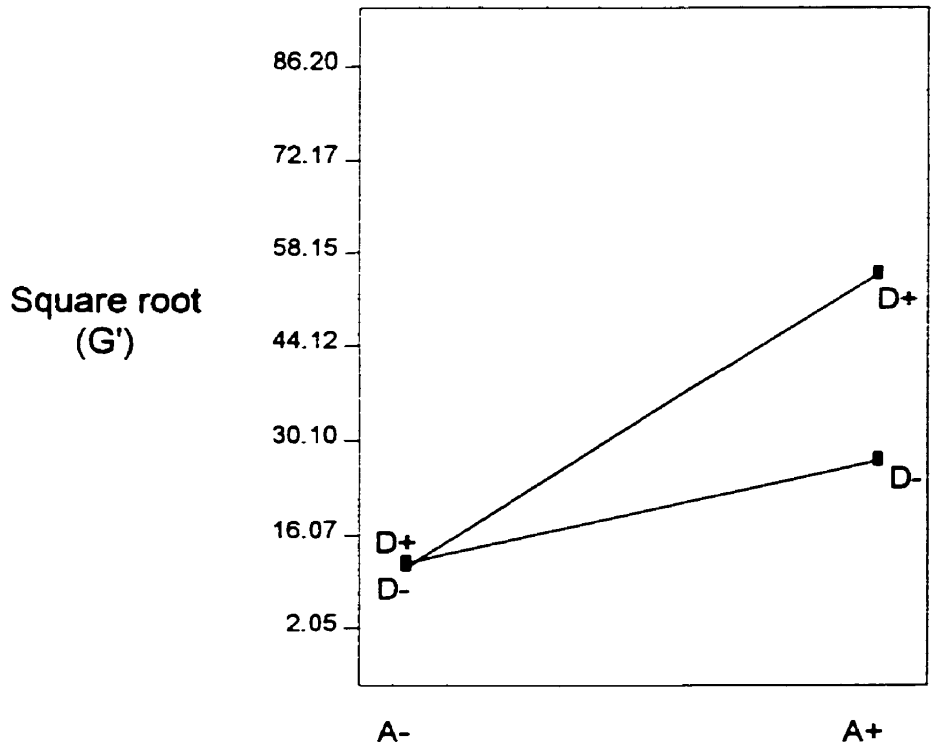
[b] Decrease in protein-polysaccharide ratio corresponds to an increase in polysaccharide concentration within the mixed system




Factors: A: Protein Concentration
" - " indicates 10% protein
" +" indicates 20% protein

B: Protein-Polysac. Ratio
" - " indicates ratio of 20
" + " indicates ratio of 100

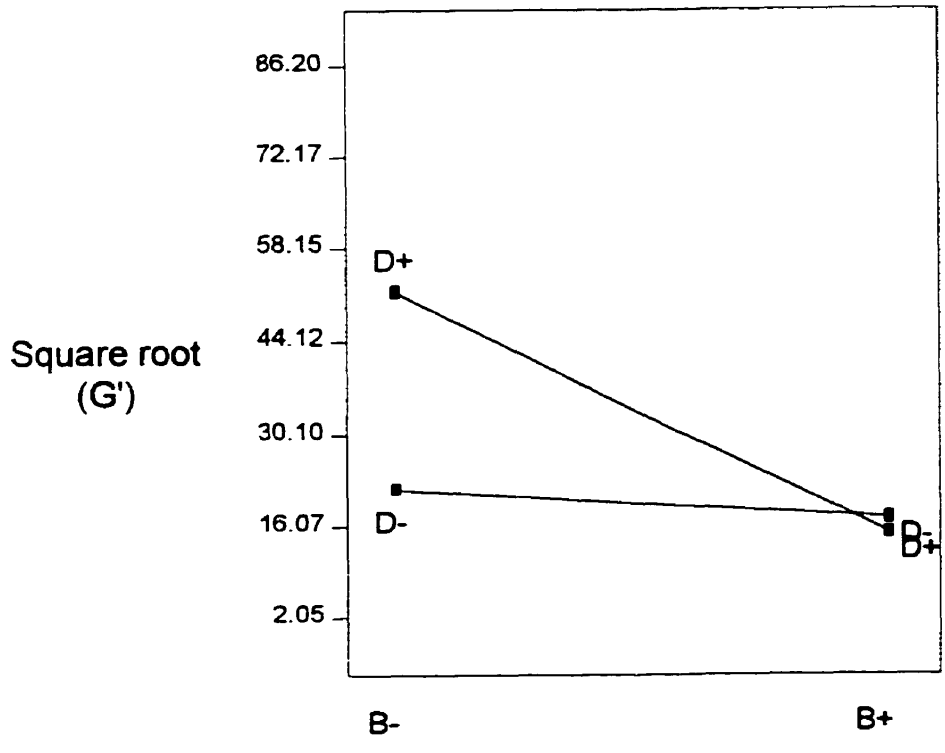
FIGURE 8. Interaction effect of protein concentration and protein-polysaccharide ratio on the loss modulus tan δ of pea protein-guar gum systems. (*Screening Experiment*)




Factors: A: Protein Concentration
" - " indicates 10% protein
" +" indicates 20% protein

D: pH
" - " indicates pH 4
" + " indicates pH 9

FIGURE 9. Interaction effect of protein concentration and pH on the storage modulus G' (Pa) of pea protein-guar gum systems. (*Screening Experiment*) Note: square root transformation was applied.



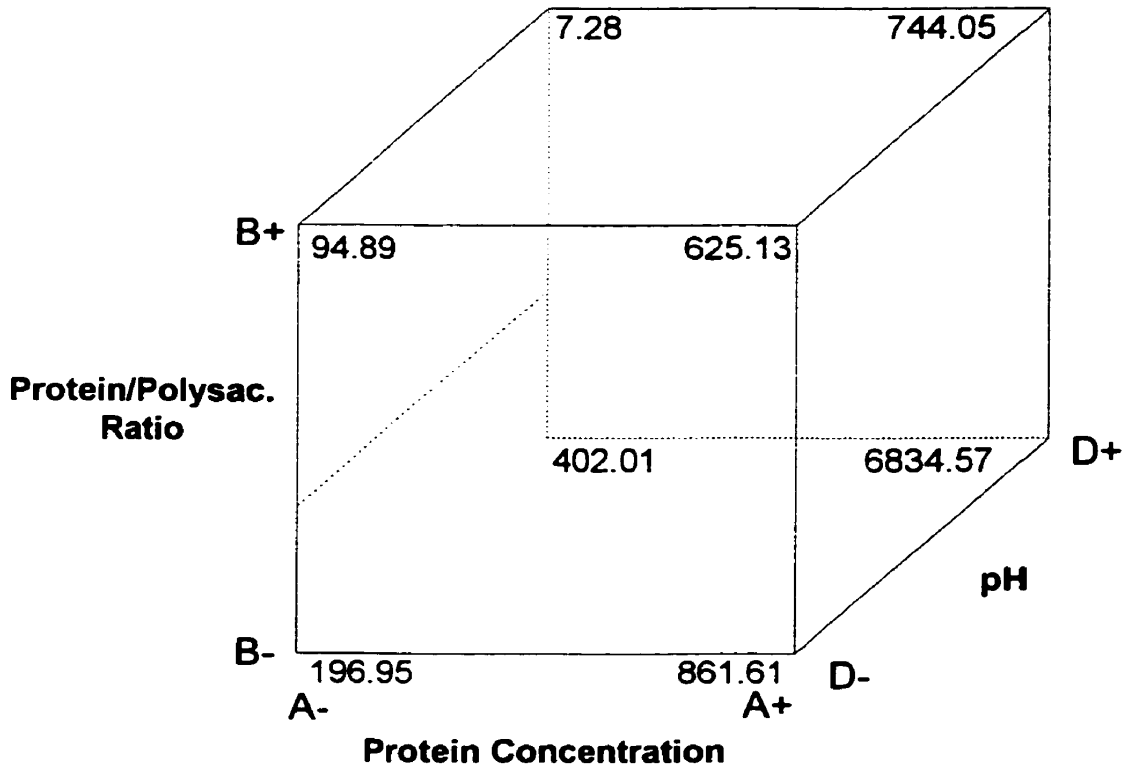

Factors: B: Protein-Polysac. Ratio
" - " indicates ratio of 20
" +" indicates ratio of 100

D: pH
" - " indicates pH 4
" + " indicates pH 9

FIGURE 10. Interaction effect of protein-polysaccharide ratio and pH on the storage modulus G' (Pa) of pea protein-guar gum systems (*Screening Experiment).* Note: square root transformation was applied.

Below the IEP, G' values remained low due to the reduced protein solubility. Insolubility was evidenced by the slight cloudiness of the composite mixture. As discussed with locust bean gum, at this pH, the potential for protein-protein interactions was great. Hence coagulation rather than gelation would have occurred. It is implied that coagulation refers to a random aggregation which occurs when protein-protein interactions predominate. Gelation on the other hand, is an ordered aggregation process which results in a well-defined network due to the balance of protein-protein and protein-solvent interactions (Hermansson 1979). Despite the cloudiness observed at a pH of 4, these mixtures remained homogenous with no formation of precipitate. As a result a mixture having high levels of both polymers, could produce opaque-colored gels with a G' as high as of 989 Pa (Appendix 6). As the pH was moved away from the IEP to a pH of 9, G' increased, reflecting improved network formation. This was most noticeable at high polymer concentrations (Appendix 6). This would indicate that protein-solvent reactions would have been favored increasing the ability of protein to solubilize and undergo gelation.

From the initial screening experiment, it is clearly evident that protein concentration, protein-polysaccharide ratio, and pH are significant contributing factors to network formation. Of the four factors tested, only protein-salt ratio was eliminated as having any contribution to the rheological parameters. Figure 11 illustrates a cube plot encompassing all significant main effects with average G' values superimposed on the eight corners. Inspection of the three-way interaction shows that highest G' values were achieved at high levels of protein concentration and pH and a low protein-polysaccharide ratio. On inspection of the associated statistical outlier plot (Appendix 9), two composite mixtures




Factors: A: Protein Concentration
" - " indicates 10% protein
"+ " indicates 20% protein

B: Protein-Polysac. Ratio
" - " indicates ratio of 20
"+ " indicates ratio of 100

D: pH
" - " indicates pH 4
"+ " indicates pH 9

FIGURE 11. Three-way interaction effect of protein concentration, protein-polysaccharide ratio, and pH on the storage modulus G' (Pa) of pea protein-guar gum systems. (*Screening Experiment*)

are shown to stray from the fitted model. This can be indicative of a synergistic occurrence (Myers and Montogomery 1995). These outlier points correspond to two composite mixtures with a 20% protein concentration, a low protein-polysaccharide ratio of 20 and a pH of 9, giving G' values of 6264 Pa and 7430 Pa. These particular mixtures were also characterized with the lowest tan delta values seen from all the experimental responses (0.109 and 0.104 respectively). Based on these highly desirable characteristics, further investigation of the inclusion of guar gum in pea protein systems was warranted. It is of interest to note that in the presence of polysaccharides, networks were established under the protein's critical point of gelation. However, when in search of the optimum conditions for an improved gel, it is felt that further experiments be conducted in a protein concentration range higher than the critical concentration point. In addition, it was decided that pH values should be set near or higher than the protein IEP to prevent instability and promote solubility within the mixture.

**Phase One - Preliminary Optimization**

The use of the central composite design was used to allow for the sequential augmentation of the 2-level fractional factorial with additional design points that would allow the fitting of a second-order surface response. Based on the previous screening results, protein concentration was set at 15 and 20%, protein-polysaccharide ratio at 15 and 75, and pH levels of 6 and 8. Protein-salt ratio was maintained at 20 as it was found that it did not contribute significantly to rheological parameters but yet it was still necessary to ensure protein solubility. Augmentation was also facilitated by the addition of axial points. Six axial points were added with $\alpha = 1.6$ chosen as the axial distance

(default value for *Design Expert V5.31)*. In a statistical design with three variables, this particular alpha value establishes data points which are equidistant from the center point.

Rheological responses are seen in Appendix 10. Based on a quadratic model, the factor exerting the most significant effect on G' ($p < 0.0001$) and tan $\delta$ ($p<0.05$) was pH. Protein-polysaccharide ratio also exhibited a significant negative effect on G' ($p < 0.05$). Appendices 11 and 12 summarize the analyses of variance for these responses. On examination of contour plots (Figures 12 and 13), it can be seen that network formation is favored (i.e. G' maximized and tan $\delta$ minimized) when the pH level is closer to the isoelectric point (i.e. from pH 8 → 6). The highest G' value, 10560 Pa, was observed at the experimental design's lowest pH value 5.32. G' values also increased as protein-polysaccharide ratios decreased (Figure 12). As seen in figures 14 and 15, the same range of protein concentration and protein-polysaccharide ratio generate much weaker gels at pH 7 than at pH 6. The trend observed with pH is similar to findings of Tolstoguzov (1995), who concluded that thermodynamic compatibility of proteins with neutral polysaccharides decreases when pH approaches the IEP of proteins. This suggests that incompatibility may play a dominant role in the present system. Given this finding, it is most probable that the mechanism by which improved gelation is rendered is through the mutual exclusion of polymers.

Protein concentration and interactions between the main effects, as seen in the screening experiment, did not contribute to rheological parameters in the preliminary optimization experiment. By narrowing the range of protein concentration and setting it above the gelation point, the influence of protein concentration was eliminated. Between

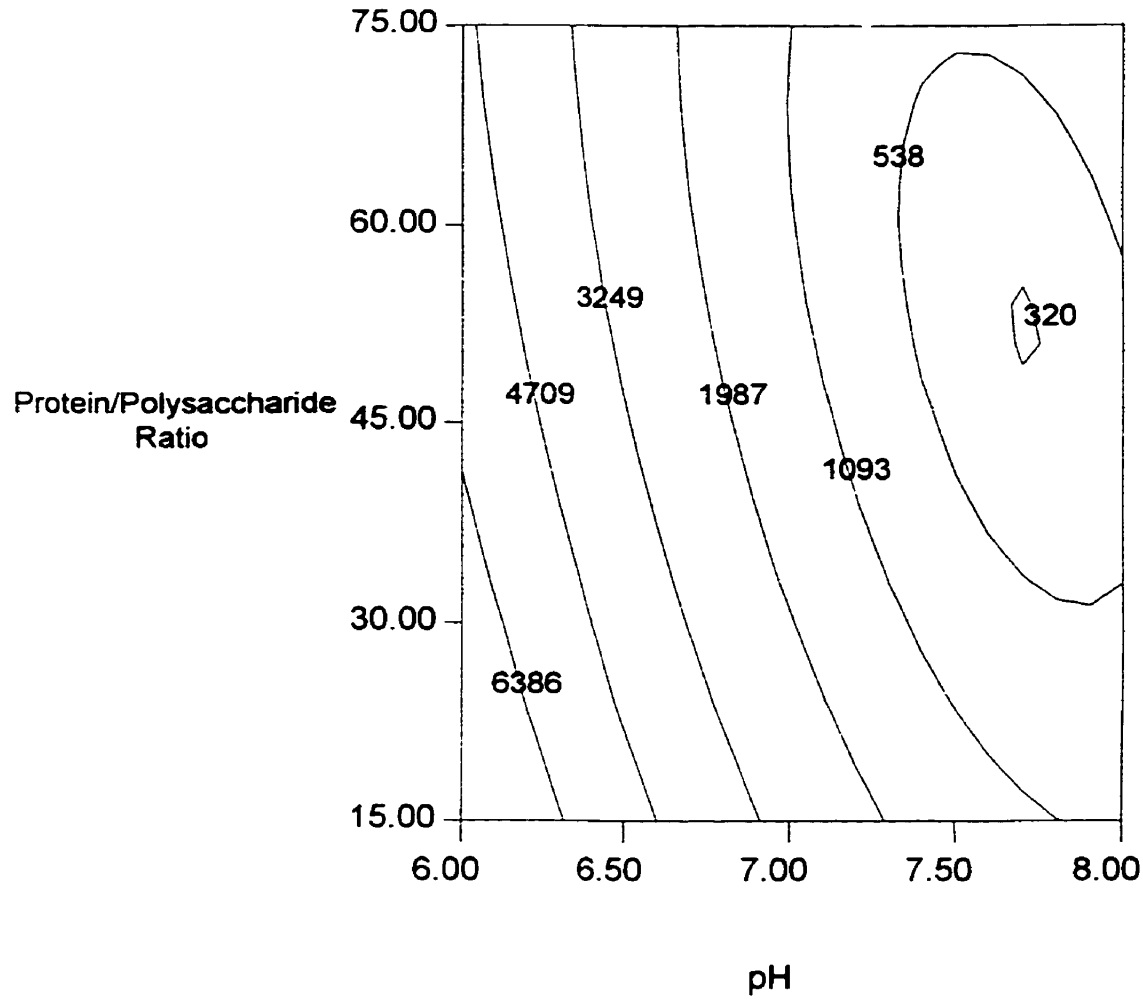



FIGURE 12. Effect of pH and protein-polysaccharide ratio on storage modulus G' (Pa) (*contour lines*) of mixed pea protein-guar gum systems at a protein concentration of 20%. (*Preliminary Optimization Experiment*)

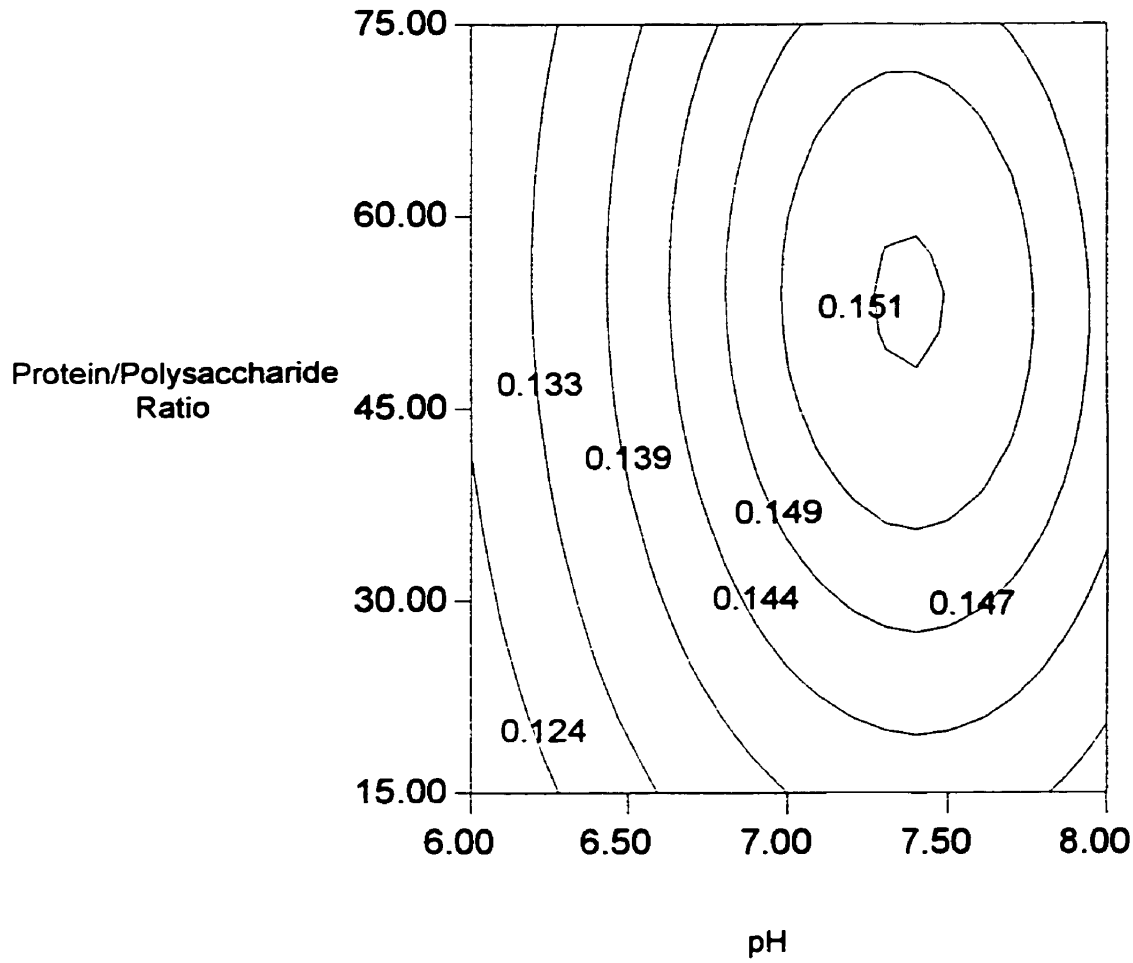



FIGURE 13. Effect of pH and protein-polysaccharide ratio on the loss modulus tan δ (*contour lines)* of mixed pea protein-guar gum systems at a protein concentration of 20%. (*Preliminary Optimization Experiment*)

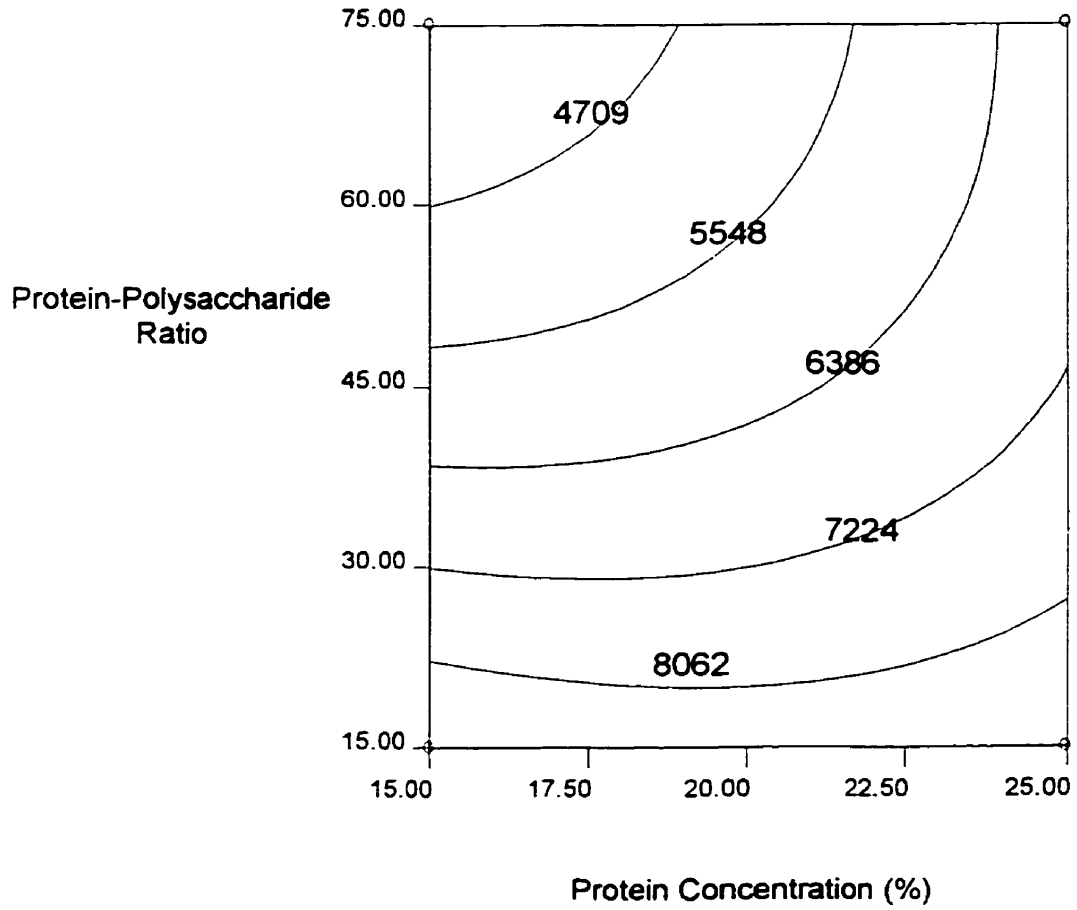



FIGURE 14. Influence of protein concentration and protein-polysaccharide ratio on storage modulus G' (Pa) (*contour lines)* of mixed pea protein-guar gum systems at a constant pH of 6 (*Preliminary Optimization Experiment*)

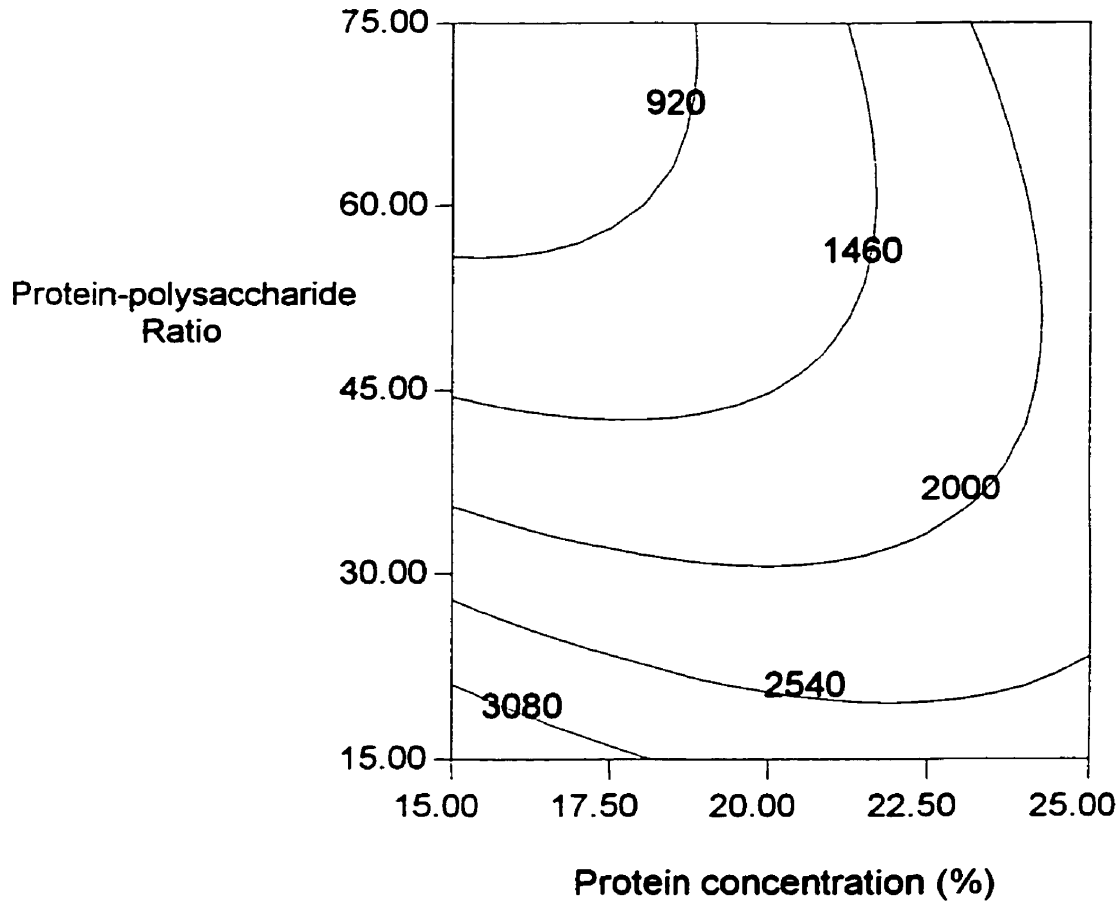



FIGURE 15. Influence of protein concentration and protein-polysaccharide ratio on the storage modulus G' (Pa) (*contour lines*) of mixed pea protein-guar gum systems at a constant pH of 7. (*Preliminary Optimization Experiment*)

levels of 15 and 25%, primary networks were always established and increasing or decreasing the level had no substantial impact on G' or tan δ (Appendices 11 and 12). However when examining outlier plots for both G' and tan δ (Appendix 13 and 14) it was observed that gelled mixtures with 28.41% protein exhibited network parameters that did not conform to the fitted model. At this protein level, a network with a high tan delta and a G' almost five times less than expected was produced. It is generally assumed that the working range for protein solubility is between 1-20 mg/ml. Such a high protein concentration may have gone beyond the solubility limits for the protein system causing it to settle out of solution. However, when examining systems comprised of only protein, a protein concentration of 28.41% did not result in a break down of gelling parameters. Therefore, the assumption that 28.41% protein is beyond solubility limits alone may not account for the undesirable G' and tan δ. However it may be that the occurrence of incompatibility and mutual exclusion may have extended the protein concentration past the solubility limits. At such levels, excess protein-protein interactions prevented the formation of a well defined crosslinked network. A closer investigation of this anomaly may have also revealed that phase separation, as a result of incompatibility, may have also contributed to the instability of the composite system.

**b. Confirmational Optimization**

Minor modification of the preliminary response surface design was achieved by way of changing axial distance to $\alpha = 1.0$. This allowed any erroneous effects to the statistical model, caused by extreme factor levels (i.e. protein-polysaccharide ratio = -5.45), to be eliminated. Rheological data for the confirmational optimization experiment can be seen

in Appendix 15. This experiment confirms the importance of pH to the gelling properties of pea protein-guar gum systems. ANOVA data (Appendices 16 and 17) indicates that pH significantly effected both G' ($p < 0.0001$) and tan delta ($p < 0.05$). Figures 16 and 17 clearly show that as pH approaches 6 , G' and tan $\delta$ values become more characteristic of a well formed network. According to ANOVA data (Appendix 14), protein-polysaccharide ratio also significantly contributed to G' ($p< 0.005$) as did its interaction with pH. The influence of the protein-polysaccharide ratio on G' was especially strong at pH values above 7.5 where the G' value dropped from greater than 1360 Pa to less than 836 Pa as the protein-polysaccharide ratio increased at a constant protein concentration of 20% (Figure 16). Still overall, as pH was lowered G' values became more desirable with higher G' values associated with lower protein-polysaccharide ratios. Similarly with tan $\delta$ (Figure 17), the influence of the protein polysaccharide ratio was greatest at about pH 7.5 but optimal values (lower tan $\delta$) were obtained at lower pH values and lower protein-polysaccharide ratios.

## 2. DSC

DSC was used to monitor changes in protein conformation upon exposure to selected test environments. Responses were recorded as thermal denaturation temperature (Td) and denaturation enthalpy ($\Delta H$) for both fractional factorial and response surface experiments (Appendix 6,10, and 15). Note that responses from phase one and phase two optimization experiments were combined for statistical analysis. The thermogram (Figure 18) for a 20% pea protein slurry, at its natural pH, was characterized by a single

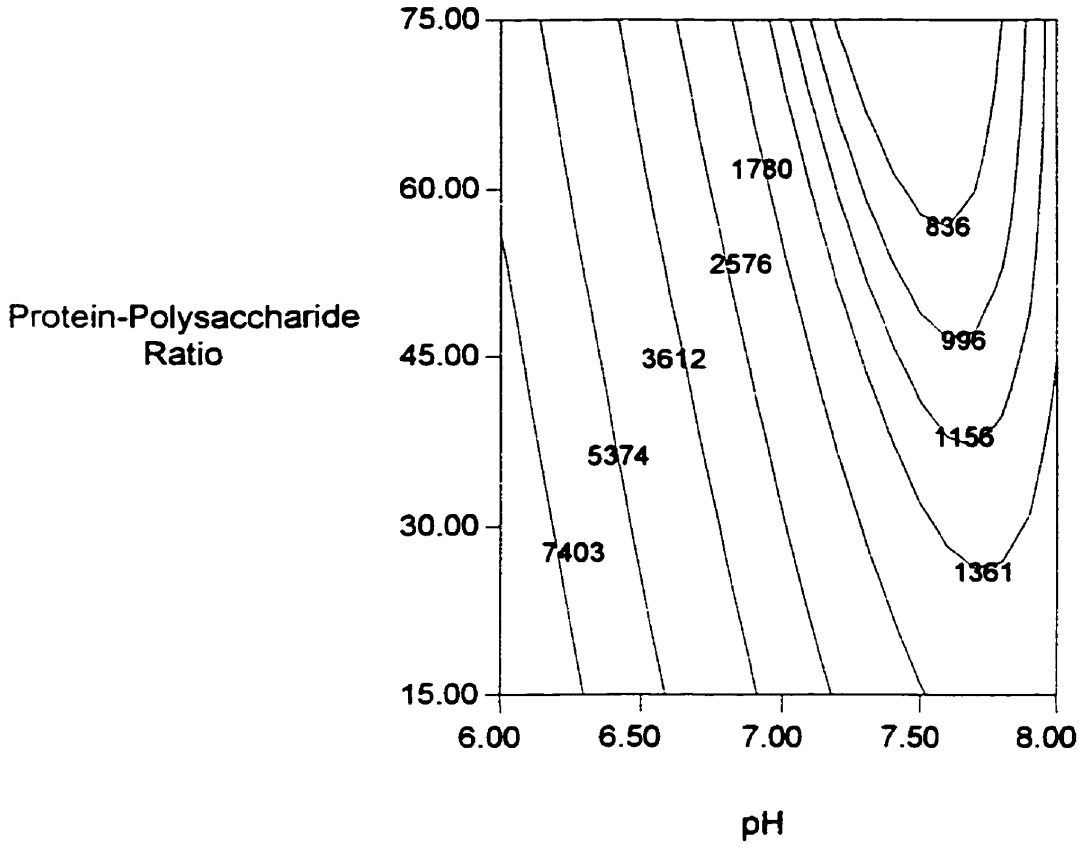



FIGURE 16. Effect of pH and protein-polysaccharide ratio on the storage modulus G' (Pa) (*contour lines*) of mixed pea protein-guar gum systems at a constant protein concentration of 20% (*Confirmational Optimization Experiment*)

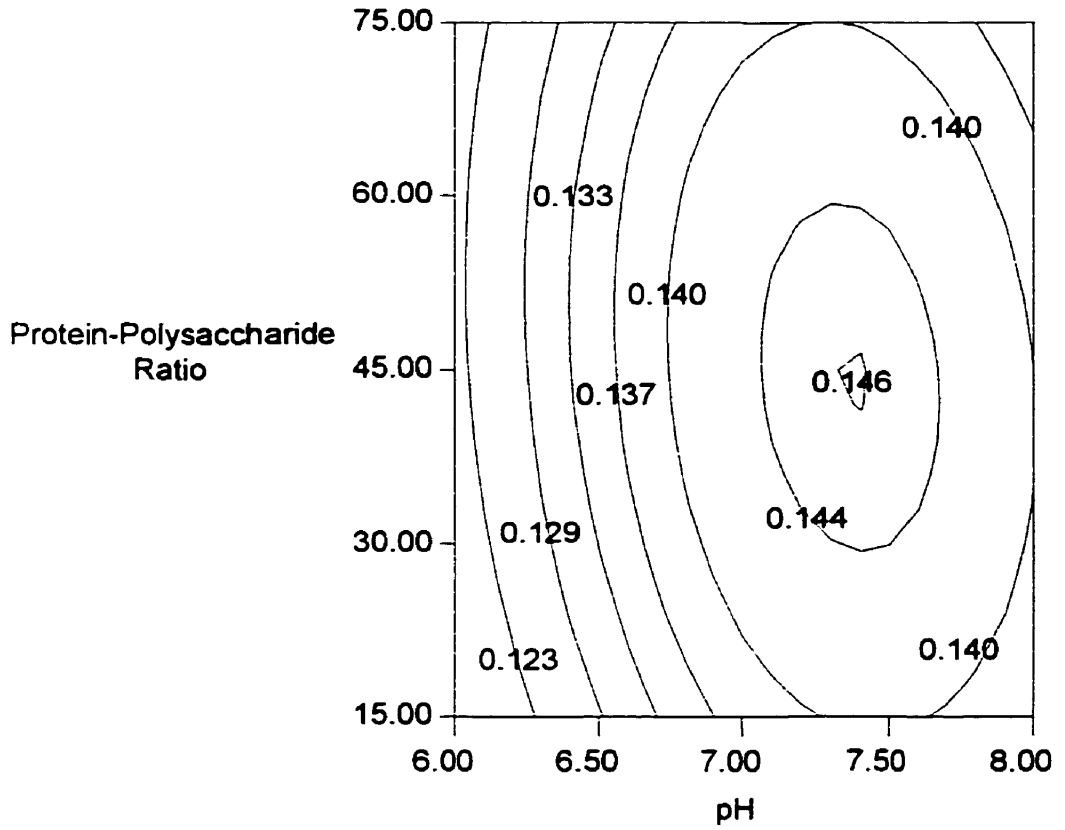



FIGURE 17. Effect of pH and protein-polysaccharide ratio on the loss modulus tan δ (*contour lines*) of mixed pea protein-guar gum systems at protein concentration of 20% (*Confirmational Optimization Experiment)*

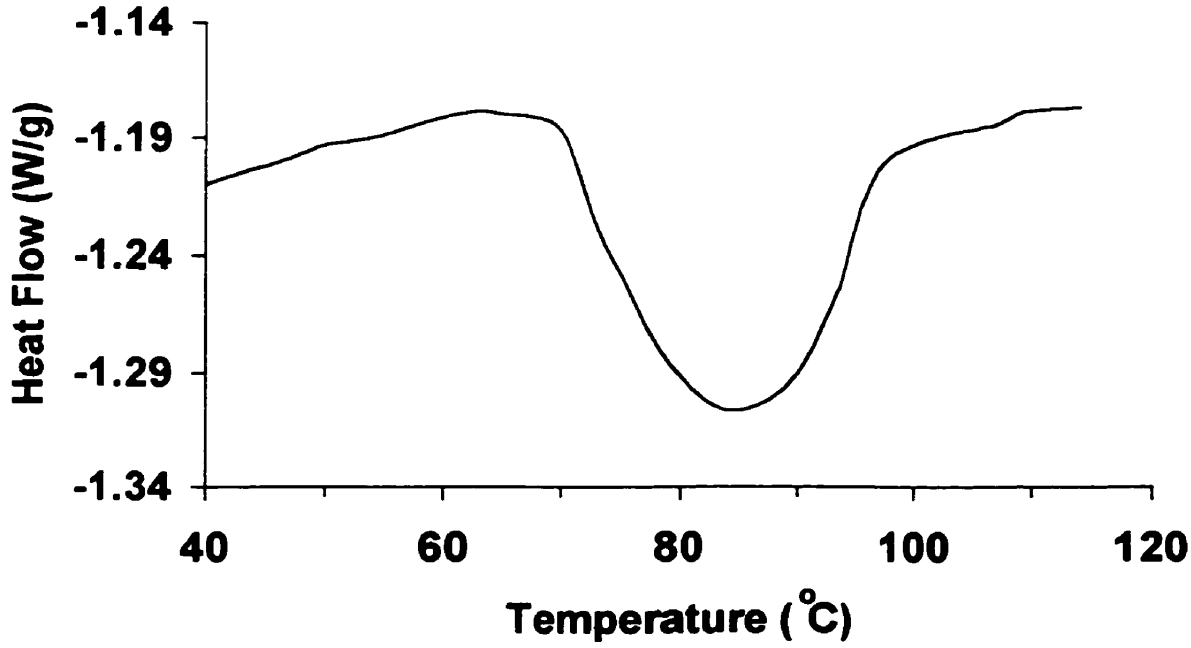



Figure 18:Thermograph of 20% (w/w) pea protein isolate at pH 7 (Td = 86.62°C, ΔH=13.02 J/g)

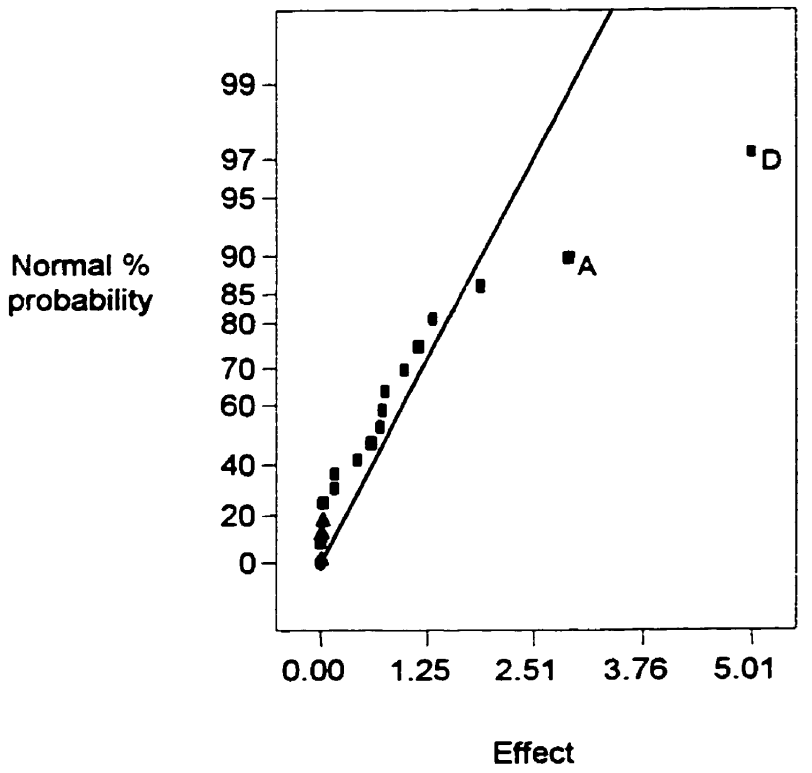
heat absorption peak with a Td and ΔH of 86.62°C and 13.02 J/g respectively. This is comparable to findings of Arntfield and Murray (1981) who observed field pea protein isolates to have a Td of 86°C and a ΔH of 3.72 cal $g^{-1}$. It must be noted that the pea protein isolate prepared according to the micelle isolation technique (Arntfield et al. 1985), often contains sufficient salt to affect the denaturation temperatures observed. Thermal analysis also revealed that the endotherm of the curve extended beyond 100°C. Since the rheometer used in this investigation had an upper limit of only 95°C, it may be suggested that network formation was not fully completed at the temperature used for gel preparation. This would result in poorer gels, characterized by lower G' values and higher tan δ values, due to the incomplete denaturation of the protein. This finding was typical of all the thermograms obtained in this study as Td values varied from approximately 85-92°C and ΔH from 8-18 J/g.

An increase in the denaturation temperature of the mixed systems may result if it is assumed that thermodynamic incompatibility is occurring between pea protein and guar gum. It is expected that exclusion of the protein in the presence of polysaccharides would decrease the effective volume available to the protein thereby making it more difficult for the protein to unfold and thus increasing the Td value. However, from both screening and optimization experiments, it was found that protein-polysaccharide ratio had no significant impact on Td or ΔH (Appendices 18-21). This is similar to findings by Burova et al (1992), who found that neutral polysaccharides did not affect the conformational stability of broad bean legumin. The lack of change in Td values, was suggested by Burova et al. (1992) to be due to the negligible change in molecular volume of the broad bean protein, as a result of its denaturation. This conclusion was made in

view of the fact that the denaturation of some small globular proteins is not accompanied by any substantial change in their molecular volume.

Factors that did contribute significantly to conformational stability, as according to factorial experiments, were protein concentration and pH (Figure 19). From the analysis of variance (Appendix 18) it was found that protein concentration had a direct positive effect on Td values ($p < 0.01$) (Table 5). Higher protein levels contribute to a shielding effect against thermal degradation giving way to increased temperatures at which denaturation takes place. More important was the significant influence of pH on Td and $\Delta H$ ($p < 0.0001$) as revealed by ANOVA data (Appendix 18 and 19). A lowering of pH into the acidic range was shown to reduce both Td and values $\Delta H$ (Table 5a-b) . At pH 4, the overall net positive charge on the pea protein was sufficient to cause a weakening of the structure allowing the protein to unfold more easily and at a lower temperature. As the pH moved up to 9, the high net charge on the protein allowed for increased protein-solvent interactions which in turn favored stabilization and delayed denaturation due to the increased solubility of the protein. Once the pH range was confined between 6 and 8, as in response surface designs, the effect of pH on conformational stability was no longer observed (Appendix 20 and 21).

Overall, the more alkaline the pH, the more stable the multicomponent system became. However according to rheology data, improved gelation took place at pH 6 rather than at higher pH values. This would suggest that protein stability was not the sole factor contributing to protein gelation. At a pH of 6, while most of the pea protein would be negatively charged a small proportion of the protein could still maintain a positive charge. As a result, the net electrostatic repulsive energy is small thereby




Factors: A: Protein Concentration B: Protein-Polysac. Ratio
C: Protein-NaCl Ratio D: pH

FIGURE 19. Normal probability plot showing significant factors with respects to the thermal denaturation temperature Td (°C) of pea protein-guar gum systems. (*Screening Experiment*)

TABLE 5. Effect of protein concentration and/or pH on (a) the thermal denaturation temperature Td (°C) and (b) the denaturation enthalpy ΔH (J/g) of mixed pea protein-guar gum systems [b] *(Screening Experiment)*

(a) Denaturation temperature, Td

| Protein Concentration (%) | pH 4 | pH 9 |
|---|---|---|
| 10 | 83.23 | 86.35 |
| 20 | 83.90 | 90.80 |

[a] Values displayed in the table are based on ranges of protein-polysaccharide and protein- NaCl ratios. Since these factors showed no statistical significance to Td, values were consequently averaged

(b) Enthalpy of denaturation, ΔH

| pH 4 | pH 9 |
|---|---|
| 6.44 | 10.22 |

[b] Values displayed in the table are based on ranges of protein concentrations, protein-polysaccharide ratios and protein-NaCl ratios. Since these factors showed no statistical significance with respects to ΔH, values were consequently averaged

contributing to greater stability and allowing for increased protein-protein interactions to occur as compared to at higher alkaline pHs (i.e. pH 8 or 9). The ability of the protein to aggregate while maintaining good stability appears to have a greater impact on the gelling capacity of the mixed system.

### 3. Solubility

Factorial and response surface analysis revealed that solubility was significantly influenced ($p < 0.0001$) by all main effects other than protein-polysaccharide ratio (Appendix 22 and 23). In terms of pH the further away the pH was from the protein's isoelectric point (IEP $\approx$ 4.5), the greater the ability of the protein to participate in protein-solvent interactions. Consequently screening experiments showed that solubility was greatest at pH 9 (Table 6). Upon visual examination, composite mixtures were denoted as "clear" at alkaline pHs, whereas mixtures at pH 4 were cloudy white in color. Response surface analysis (Appendix 23) also showed that pH had a significant positive effect on solubility ($p < 0.0005$). Note that the statistical analysis of the two optimization studies with respects to solubility, were done in combination. At pHs between 6 and 8 mixtures were slightly cloudy but maintained a slight yellow color. In all such cases, mixtures remained homogenous in nature with no phase separation or precipitation observed. Figure 20 clearly shows the increase in solubility as pH becomes more alkaline.

Analysis of screening and optimization experiments also revealed that protein concentration also had a significant effect on solubility ($p < 0.0001$) (Appendix 22 and 23). Reasons are similar to those seen previously with locust bean gum. Based on both

TABLE 6. . Effect of protein concentration and pH on the protein solubility (g/100g) of mixed pea protein-guar gum systems[a,b]. (*Screening Experiment*)

| Protein Concentration (%) | pH 4 | pH 9 |
|---|---|---|
| 10 | 5.38 | 10.91 |
| 20 | 8.19 | 19.37 |

[a] Values displayed in table are at a protein-NaCl ratio of 10.

[b] Values displayed in table are based on ranges of protein-polysaccharide ratios. Since this factor showed no statistical significance to protein solubility, values were consequently averaged

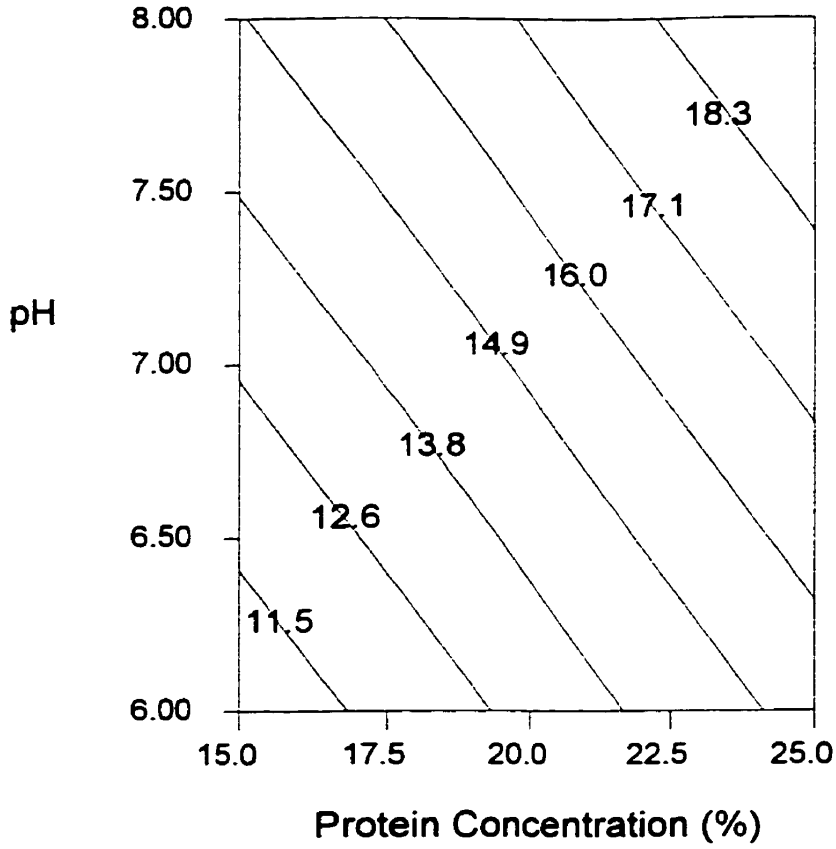



FIGURE 20. Effect of pH and protein concentration on the protein solubility (g/100g) (*contour lines*) of mixed pea protein-guar gum systems at a constant protein-polysaccharide ratio of 45. (*Combined Optimization Experiment)*

sets of experiments, a protein range anywhere from 10 to 25% was observed to be within the solubility limits of the pea protein, since mixtures remained homogenous with no precipitation of the protein. Therefore, as protein concentration within the mixed system increased, the amount of protein being solubilized also increased. This positive relationship between protein concentration and solubility, as seen in both screening and optimization experiments, is seen in Table 6 and Figure 20 respectively. However, a deviation from this trend was observed at one of the response surface axial points. The high protein concentration of 28.41%, as discussed previously, showed a significant loss in solubility (6.35 g/100g), such that it was excluded from the statistical model (Appendix 24). A similar loss in functionality was also seen in rheological experiments where a highly significant decrease in gelling capacity was seen for this particular sample. This supports the notion that some form of sedimentation must have taken place which inhibited network formation.

It is understood that thermodynamic incompatibility between polymers can be clearly identified by the occurrence of phase separation. Within the present research, phase separation was not visually observed nor quantitatively assessed by solubility measurements. However, the absence of phase separation does not negate the occurrence of thermodynamic incompatibility. According to Tolstoguzov (1995) synergistic effects as a result of thermodynamic incompatibility can take place in single-phase mixtures where, due to mutual exclusion, each polymer occupies only part of volume of the bulk solution. Therefore the lack of effect on solubility by protein-polysaccharide ratios may still possibly be attributed to the incompatibility between the polymers. The apparent lack of interaction between pea protein and guar gum would impede any effect that guar

gum has on protein conformation. Changes in solubility depend on some form of alteration in conformation or change in the balance of protein-protein to protein-solvent interactions and these factors were unaffected by the presence of guar gum.

**4. Numerical and Graphical Optimization**

To determine the conditions required for improved gelation, a simultaneous optimization of G' and tan δ was made using the combined data from preliminary and confirmational optimization experiments (phases 1 and 2). Numerical and graphical optimization will optimize any combination of one or more goals. The goals set may apply to either factors or responses. As such, constraints (or goals) were put in place for the factors: protein concentration, protein-polysaccharide ratio and pH and the responses: G' and tan δ. Since the primary role of DSC and solubility data was to help explain and clarify rheological results, they have not been included in this optimization as defining factors of network formation. Constraints for factors were based on the maximum axial points (i.e. alpha value ($\alpha$) = 1.6) used in the prior response surface experiments (Table 7). Since protein-polysaccharide ratio displayed an erroneous axial point value at $\alpha$= -1.6 (i.e. -5.45), the constraint was redefined to 10. The goal for G' was set to 20 000 Pa in order for predicted networks to be characterized with the highest G' response possible. Gels characterized with a G' greater than 10 kPa have been evaluated as being acceptable well-defined elastic networks (Mitchell 1980). A target value of 0.05 was set for tan δ responses, however values between 0.01 and 0.11 were deemed as acceptable. Note that the criteria for graphical optimization is based on the *range* of factor and response values rather than target values as seen in numerical optimization.

| CONSTRAINTS | | | |
|---|---|---|---|
| **Name** | **Goal** | **Lower Limit** | **Upper Limit** |
| Protein Concentration (%) | w/in range | 11.59 | 28.41 |
| Prot-Polysac. Ratio | w/in range | 10.00 | 95.45 |
| PH | w/in range | 5.32 | 8.68 |
| G' (Pa) | maximize | 10000 | 20000 |
| Tan δ | target 0.05 | 0.01 | 0.11 |

| SOLUTIONS (*Output from Numerical Optimization*) | | | | | | |
|---|---|---|---|---|---|---|
| # | Protein Conc (%) | Prot-Poly Ratio | pH | G' (Pa) | Tan δ | Desirability (%) |
| 1 | 11.64 | 10.60 | 5.32 | 16399 | 0.050 | 80.0 |
| 2 | 11.61 | 11.75 | 5.32 | 16240 | 0.050 | 78.5 |
| 3 | 11.59 | 12.99 | 5.32 | 16060 | 0.051 | 76.9 |
| 4 | 15.26 | 10.00 | 5.32 | 16005 | 0.066 | 66.0 |
| 5 | 16.88 | 10.00 | 5.32 | 15906 | 0.072 | 61.5 |
| 6 | 28.41 | 46.31 | 5.32 | 15063 | 0.073 | 55.8 |
| 7 | 28.41 | 71.00 | 5.32 | 14348 | 0.069 | 54.7 |

Table 7. Predicted combinations of factors (protein concentration, protein-polysaccharide ratio, and pH) and responses (G' and tan δ) characterizing optimized gelled networks based on set constraints within numerical optimization.

Numerical optimization displays numerical predictions (solutions) that simultaneously satisfy the constraints from both multiple factors and responses. Such optimization procedures pinpoint solutions with the greatest desirability. Table 7 indicates that the most desirable network (solution #1) was produced at a protein concentration of 11.64%, at a protein-polysaccharide ratio of 10.60 (corresponding to a polysaccharide concentration of 1.10%), and a pH level of 5.32. This acceptable network was defined by a G' of 16 399 and a tan delta of 0.050. This illustrates that in order to obtain a well-defined network (as according to the criteria set), protein concentration, protein-polysaccharide ratio and pH need to be at a minimum level. Note that a minimum protein-polysaccharide ratio corresponds to a relatively high concentration of polysaccharide. Decreasing the presence of polysaccharide to a minimal level within the system (i.e. increasing the protein-polysaccharide ratio) requires a maximum increase in protein concentration in order to maintain an acceptable network (Table 7, solution #7). However in this situation, a compromise in both the storage modulus and tan delta response is evident. Previous rheological study demonstrated that extremely high values of protein concentration (i.e. 28.41 %), associated with high polysaccharide levels, were undesirable for network formation since it created an unstable system. What is also apparent is the importance of pH in these mixed systems. All solutions presented indicate that a pH of 5.32 is required to generate desirable networks. Since no solutions showed any other acceptable pH values, it is assumed that any deviation from pH 5.32 would considerably diminish the desirability of the networks.

Graphical optimization gives a better indication of the actual *range* of factor levels permissible to obtain acceptable response values. Graphical optimization displays the

area of feasible response values within the factor space. The area that does not fit the optimization criteria remains unshaded. Areas within the factor space that are shaded will indicate that the multiple constraints on the responses have been satisfied. Shaded areas are enclosed by contour lines illustrating the limits imposed by response constraints. It must be noted that graphical optimization does not differentiate between the desirability of the networks formed as in numerical optimization.

Graphical optimization supports findings of above and confirms that a pH of 5.32 allows for the most conducive environment to form satisfactory networks. This pH allows for the broadest range of both protein and polysaccharide levels (Figure 21). At a pH of 5.32, networks can be formed with protein concentrations anywhere between 11.59 and 28.41% (Figure 21). However to generate an acceptable gel at a minimal protein concentration of 11.59%, protein-polysaccharide ratios below 60.63 are required (Figure 22). This corresponds to having polysaccharide concentrations only above 0.19%. As seen in Figure 22, as pH approaches 6.07, the level of polysaccharide required within the system increases (i.e. protein-polysaccharide ratio decreases).

To achieve a desirable gel at pH 5.32, with the least amount of guar gum (i.e. protein-polysaccharide of 95.45) a minimum protein concentration of 20% must be met (Figure 23). This minimum protein requirement becomes higher as higher pH values are used. Previous analysis of pea protein-guar gum systems, has shown that protein levels above 25% are undesirable due to lack of protein solubility. High concentrations of guar gum are therefore seen as a benefit, since it allows protein concentrations to be kept within a workable range (i.e. 10-25%).

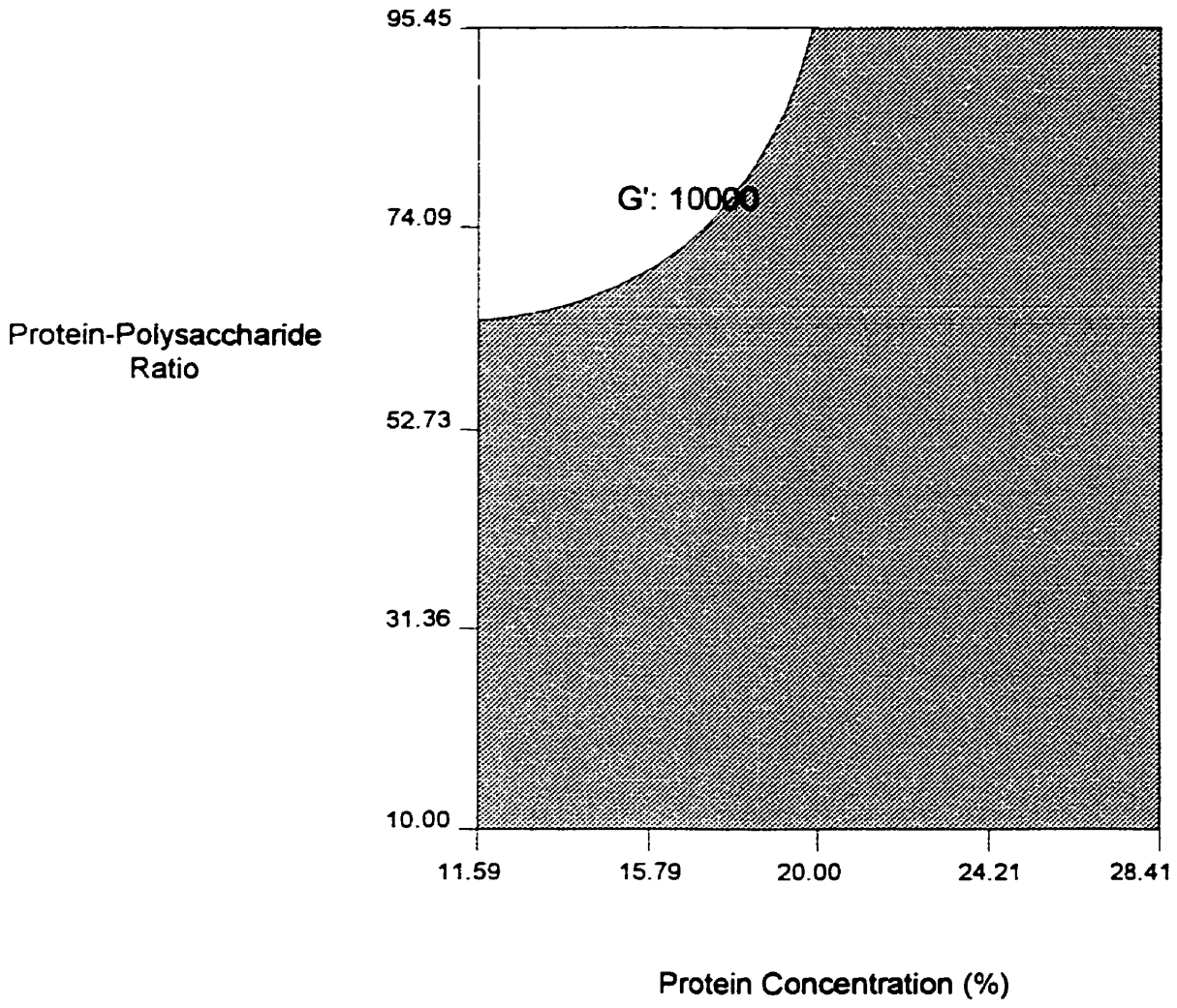



Figure 21. Graphical projection of optimized gelled networks from pea protein-guar gum systems as a function of protein-polysaccharide ratio and protein concentration at a constant pH level of 5.32.

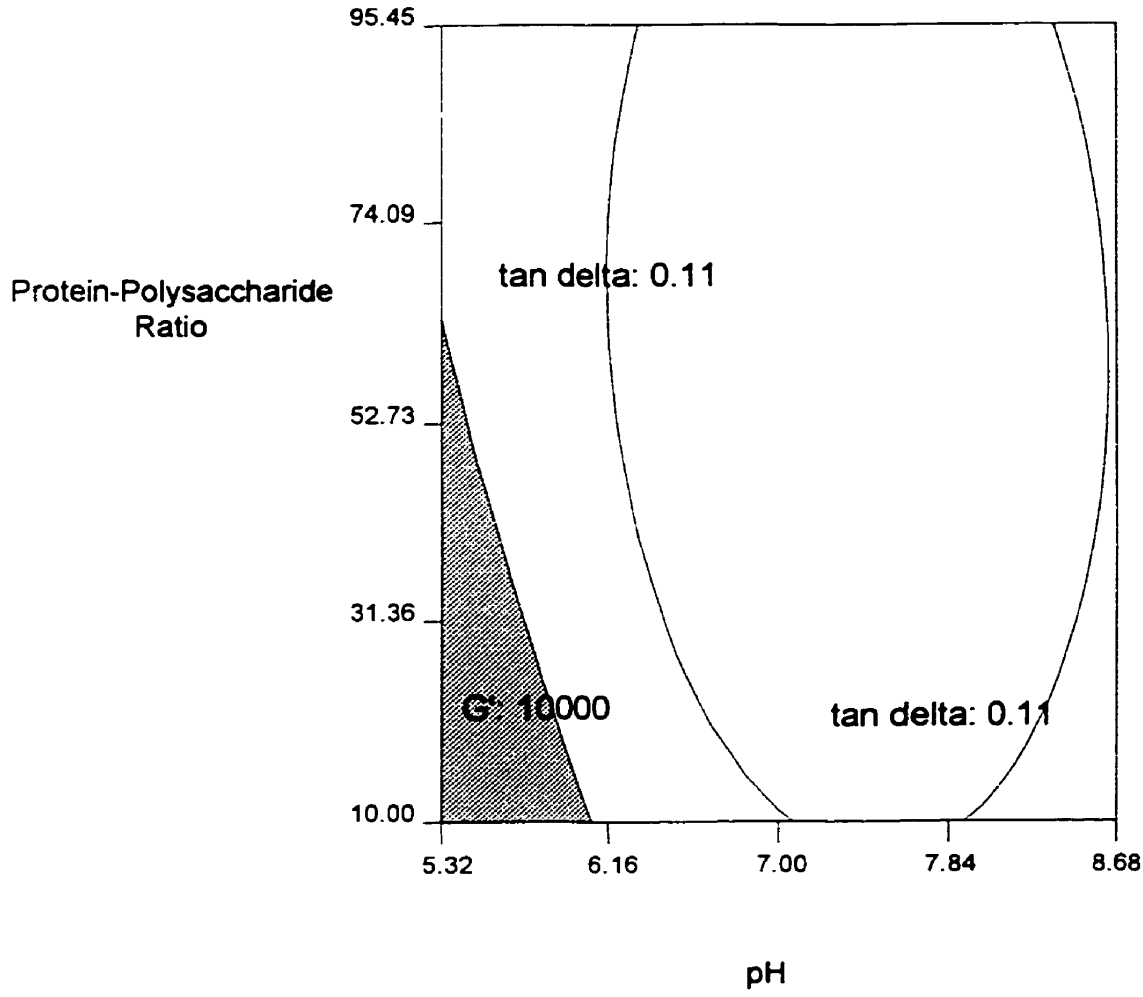



Figure 22. Graphical projection of optimized gelled networks from pea protein-guar gum systems as a function of protein-polysaccharide ratio and pH at a constant protein concentration of 11.59%.

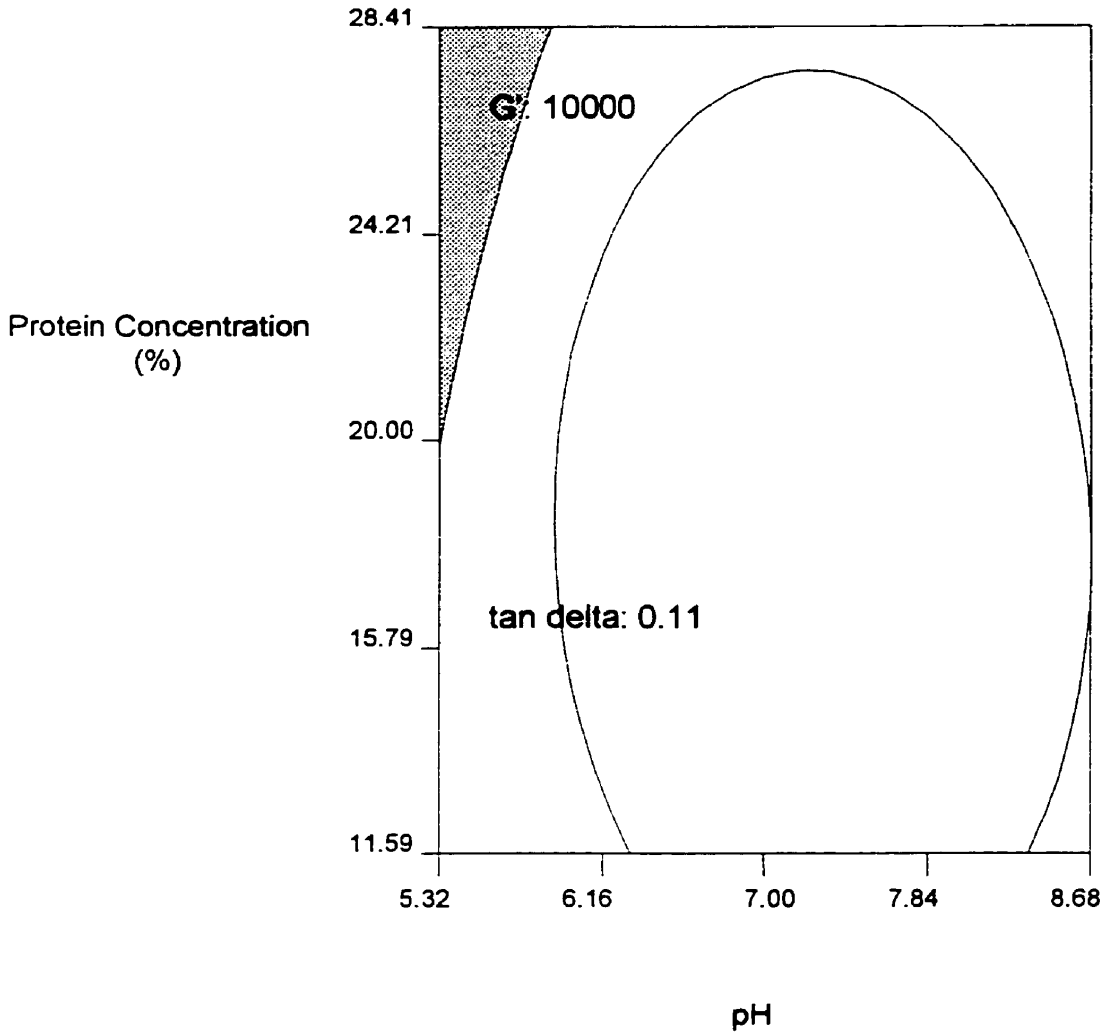


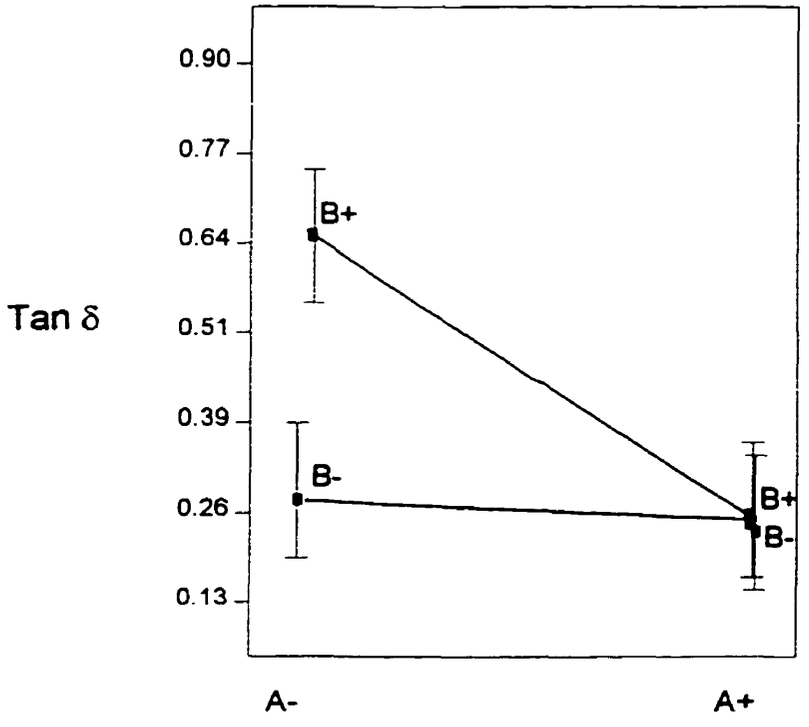

Figure 23. Graphical projection of optimized gelled networks from pea protein-guar gum systems as a function of protein concentration and pH at a constant protein-polysaccharide ratio of 95.45.

## C. CARRAGEENAN SYSTEMS

### 1. Rheology

#### a. Screening Experiment

Original data and experimental conditions tested during the screening experiment of carageenan systems are given in Appendix 25. Results from the analysis of variance (Appendix 26 and 27), showed that protein concentration, protein-polysaccharide ratio, their interaction (i.e. AB) and pH exhibited significant effects on both G' and tan δ ($p < 0.0001$). It must be noted that for the storage modulus responses, a square root transformation was used to generate a model that better described the data. As described earlier, the increase in G' with protein concentration has been attributed to the number of intermolecular protein-protein interactions commonly referred to as crosslinks or junction zones (Smith 1994). As the protein concentration increased, G' values increased due to the greater probability of intermolecular contact. In addition to a main effect, the effect of protein concentration on tan δ responses also depended on the level of polysaccharide. Figure 24 illustrates how low values of tan δ were consistently associated with all protein concentrations when protein-polysaccharide ratios were low. From all the responses observed, a mixture composed of 20% protein, a low protein-polysaccharide ratio of 20 and a pH of 9, appeared to give the highest G' (4750 Pa) and lowest tan δ (0.127) values. At low protein concentrations (i.e. 10%), G' values remained low and tan δ values high, regardless of the protein-polysaccharide ratio. This observation is in contrast to what was seen in guar gum systems, where even when the protein concentration was below the critical gelation point (i.e. 10%) composite systems would produce a thin elastic gel when in the presence high guar gum concentrations. This ability to form gels below




Factors: A: Protein Concentration
" - " indicates 10% protein
" +" indicates 20% protein

B: Protein-Polysac. Ratio
" - " indicates ratio of 20
" + " indicates ratio of 100

FIGURE 24. Interaction effect of protein concentration and protein-polysaccharide ratio on the loss modulus tan δ of pea protein-κ-carrageenan systems. (*Screening Experiment*)

the critical protein concentration was attributed to the fact that mutual exclusion of the polymers was taking place. With the carrageenan systems however, all mixtures with low protein levels remained in a liquid progel-like state. Therefore, it appeared that a minimum protein concentration was necessary before the influence of carrageenan concentration could be seen. Significant increases in G' were not seen until a protein concentration of 15%, which may indicate that the protein gelling threshold had then been reached.

As with the guar gum systems, pH seems to play a distinctive role in determining the characteristics of the resulting network. At a pH of 4, pea protein carries a net positive charge since it is below its isoelectric point. It is also known that carrageenan retains a strong negative charge over the normal pH range of 3 to 10 (Burova et al. 1992). So it may be assumed that the opposite charges exhibited by the two polymers result in compatibility and subsequent interaction. At acidic conditions it was observed that mixtures were not only accompanied by cloudiness, as seen with guar gum systems, but also by precipitation. The interaction among the whole unit of the polymers would result in the association of a large colony and subsequent precipitation in the form of insoluble complexes (Tolstoguzov 1995). The formation of these complexes was a characteristic difference between carrageenan and guar gum systems. However the presence of these complexes seemed to have a negative impact on both elasticity and network properties. Tan $\delta$ values at pH 4 were significantly higher than that at pH 9. This indicates that the gel was presumably strengthened through aggregation rather than network formation since gels formed by aggregation are less structured (Cai 1996). Since the improved gelation of biopolymer systems is the desired outcome pH levels below the isoelectric

point were avoided in subsequent investigation. As pH values were raised above the IEP, mixtures became increasingly translucent and this resulted in more favorable rheological parameters. Interaction graphs (Figures 25 and 26) illustrate how high protein and polysaccharide levels contributed to higher G' values when the higher pH was used. At a pH of 9 the protein isolate has a large net negative charge. Since carrageenan also carries a negative charge, it could be postulated that repulsion and incompatibility between the polymers would occur. Consequently, the improved gelation would presumably be due to the mutual exclusion of pea protein and carrageenan into their own domains thereby increasing the protein's effective concentration.

**b. Preliminary Optimization**

Utilizing the significant factors found in the factorial experiment a response surface design was applied to the mixed system, (Appendix 28). Protein concentration was adjusted to a range that would be above the necessary critical concentration for gel formation for pea and pH levels were held above the IEP in the range of 6 to 8. The presence of salt within the system was still required in order to aid in protein solubility. However, the protein-salt ratio was kept constant at 20, since this factor was determined to have an insignificant effect on gelling parameters as seen in the screening experiments.

Based on the analysis of variance (Appendix 29 and 30) for these conditions, protein-polysaccharide ratio was the only significant factor affecting the storage modulus ($p < 0.005$) while pH and protein concentration significantly affected tan $\delta$. As seen in Figure 27, mixtures consisting of low protein-polysaccharide ratios resulted in increased G' values. For example, composite systems with a protein-polysaccharide ratio of 15 gave

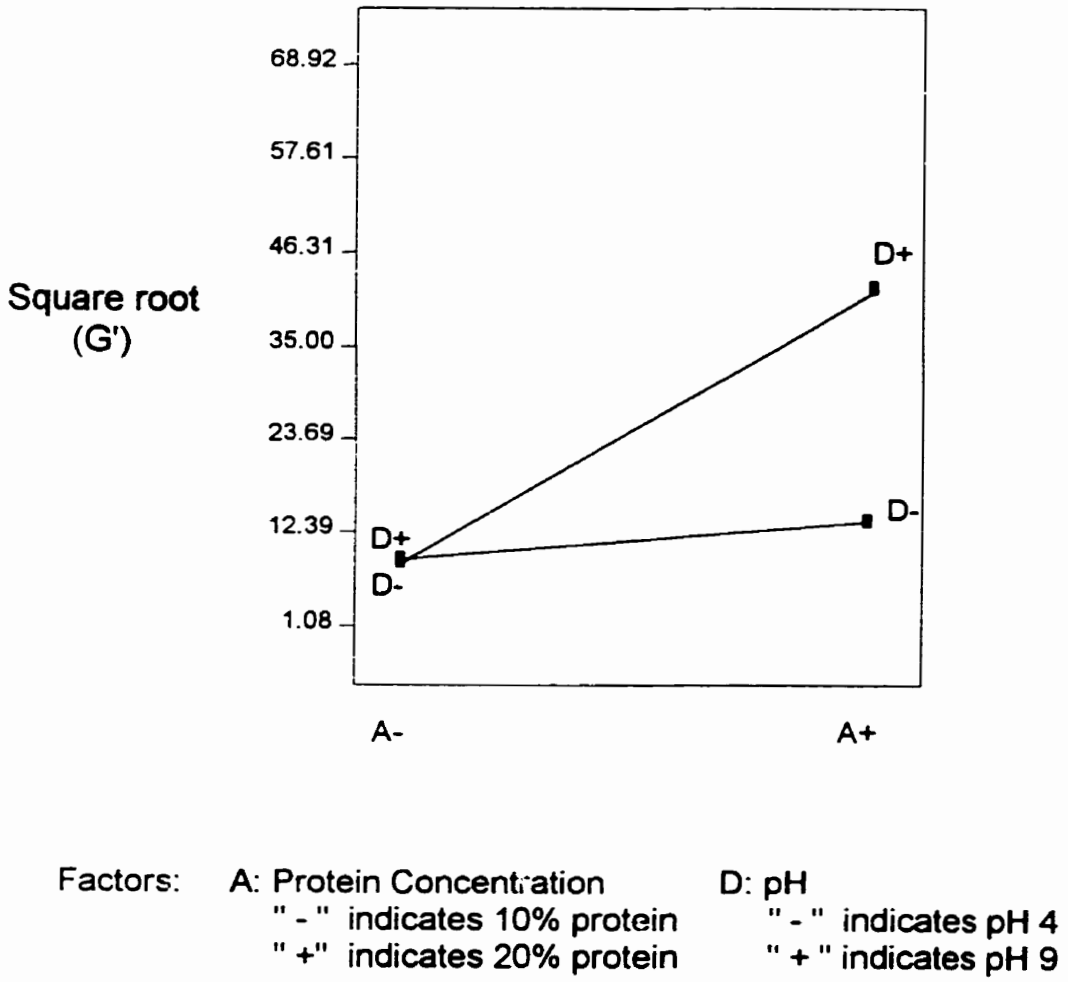



FIGURE 25. Interaction effect of protein concentration and pH on the storage modulus G' (Pa) of pea protein-carrageenan systems (*Screening Experiment*). Note square root transformation was applied.

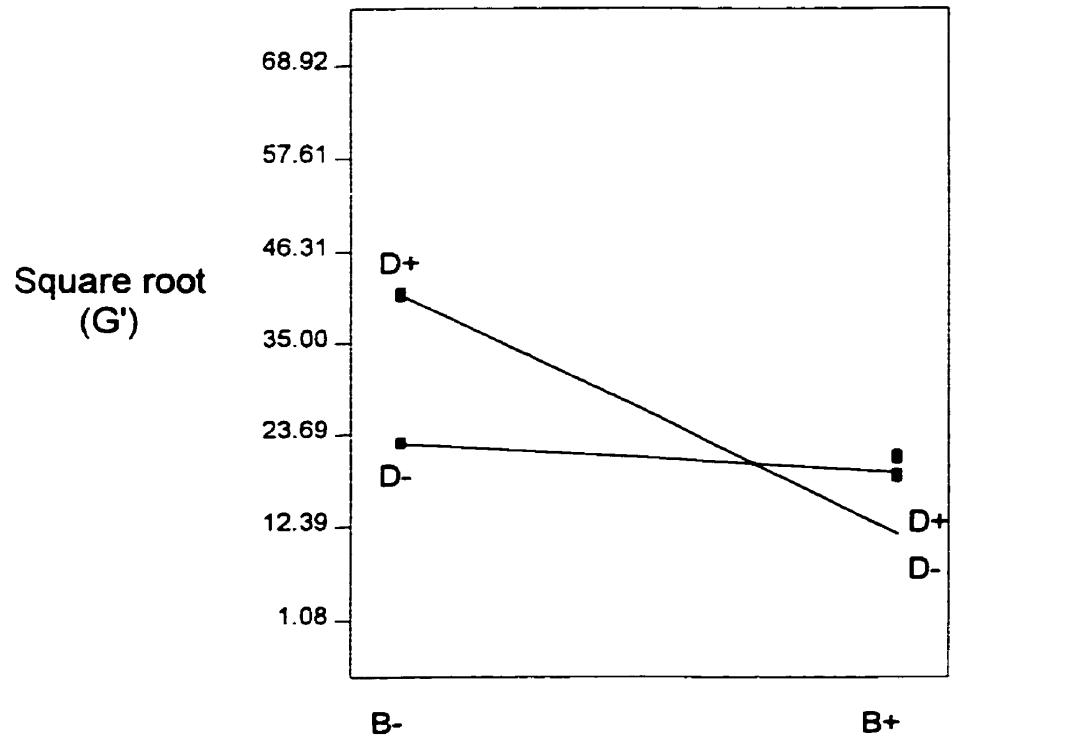



FIGURE 26. Interaction effect of protein-polysaccharide ratio and pH on the storage modulus G' (Pa) of pea protein-carrageenan systems (*Screening Experiment*). Note: square root transformation was applied)

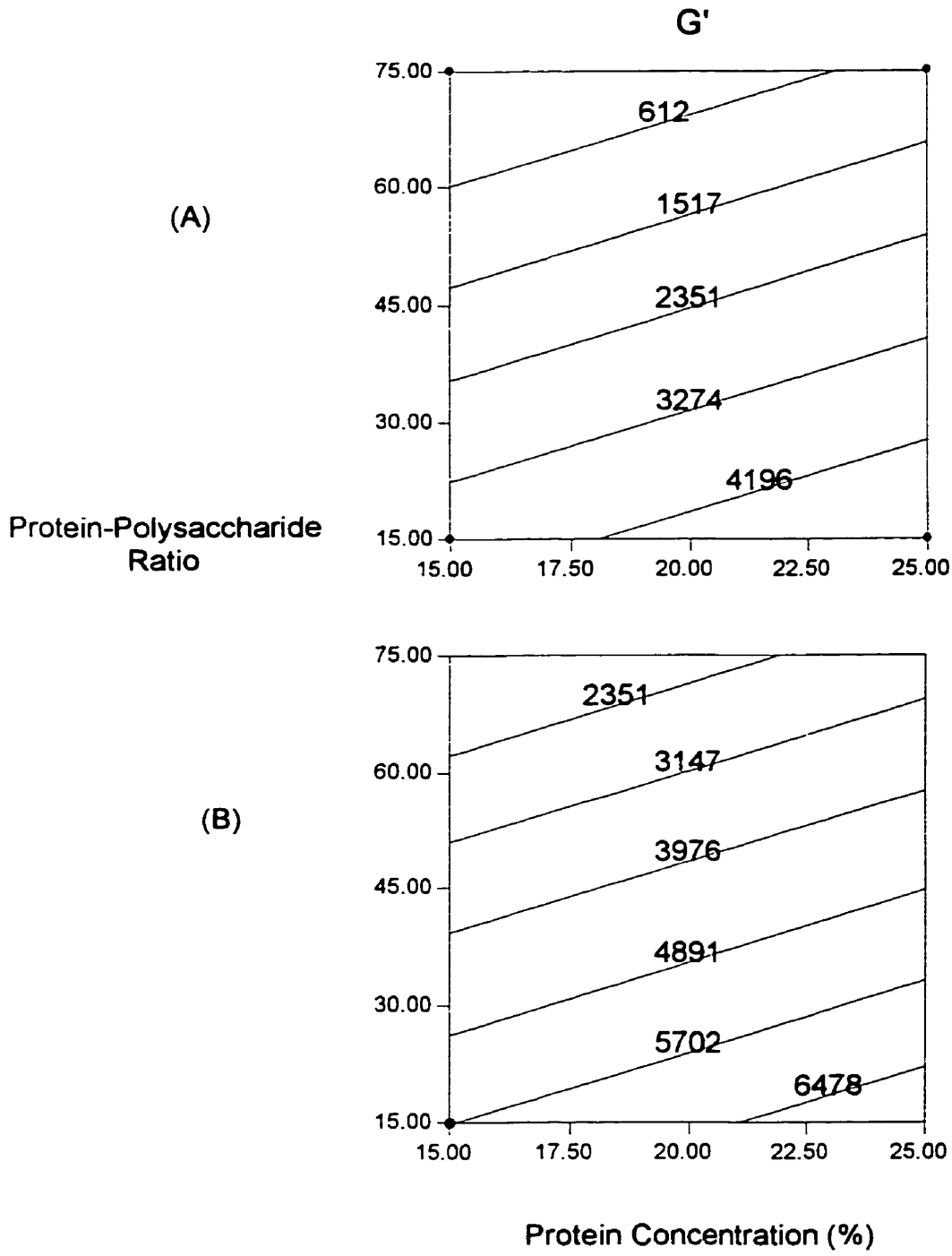



Figure 27. Effect of protein-polysaccharide ratio on the storage modulus G' (Pa) (*contour lines*) of mixed pea protein-κ-carrageenan systems at (A) pH 6 and (B) pH 8. (*Preliminary Optimization Experiment*)

the highest G' responses measured.

At pH 6 and 8, mean values for G' were 7640 Pa and 9135 Pa, respectively and were not significantly different. Tan δ values however, were significantly different at varying pHs, with average values of 0.147 and 0.104, for pH 6 and 8 respectively. Thus it is evident that mixtures at a pH of 8, where more favorable loss tangent values were obtained, resulted in superior gelled networks. One explanation for this improved gelation, above pea protein's IEP, could be thermodynamic incompatibility. In this case, the net negative charges of pea protein and carrageenan would bring about repulsion and subsequent movement of each polymer into its own domain. Besides the possibility of thermodynamic incompatibility, an alternative interpretation of the data can be explained by the occurrence of complexes (Tolstoguzov 1995). This interaction is attributed to the non-uniform distribution of cationic and anionic amino acid residues in the protein. The separation of charged groups gives rise to micro-regions of net positive charge which can interact with the anionic polysaccharide even though the net charge on the entire protein is negative. Sulphated polysaccharides, because they have a higher charge density, are particularly capable of forming soluble complexes with globular proteins at pH values above the protein IEP (Ipsen 1995). These micro-regions on the protein, can provide sufficient interaction space to accommodate polysaccharide molecules. This type of interaction is most probable when the protein chain has been unfolded by some kind of force, such as heat. Evidence supporting this binding mechanism can be seen with the rheological response of mixtures containing 28.41% protein at a pH of 7. As discussed previously with guar gum, mixtures with this high concentration of protein resulted in a sharp drop in G' and tan δ values. This was attributed to the occurrence of mutual

exclusion of macromolecules. However in the case of carrageenan, there was no extreme deterioration in gelling properties. Protein-carrageenan mixtures at this high protein level resulted in a G' of 3596 Pa. This may support the notion that exclusion between the polymers did not occur with carrageenan systems but rather the evidence of gel formation indicates the occurrence of complexing.

**c. Confirmational Optimization**

The important effects that emerged from the confirmational response surface study (Appendix 31) of pea protein-carrageenan systems were quite similar to those seen with the preliminary study. The main effects, protein-polysaccharide ratio and pH, had the greatest impact on rheological parameters ($p < 0.0001$) (Appendix 32 and 33). Protein concentration was also shown to have an effect on both G' and tan $\delta$ ($p < 0.05$), though not as significant. As seen throughout the rheological study, protein concentration will invariably have an effect on rheological parameters as it is the protein that forms the basic and foundational structure of the gel. The confirmational optimization experiment demonstrated that G' was dependent on pH, a factor which was important in the screening study but was not seen as significant in the preliminary optimization experiment. Based on this experiment, it can be suggested that the mechanism by which carrageenan encourages gelation of pea proteins, is heavily dependent upon the pH.

As seen in phase one, G' values were highest with mixtures composed of a low protein-polysaccharide ratio of 15 whether at pH 6 or 8. A response surface contour plot illustrates quite clearly how at low protein-polysaccharide ratios, G' and tan $\delta$ values are most favorable (Figures 28). The fact that higher polysaccharide concentrations (~1%)

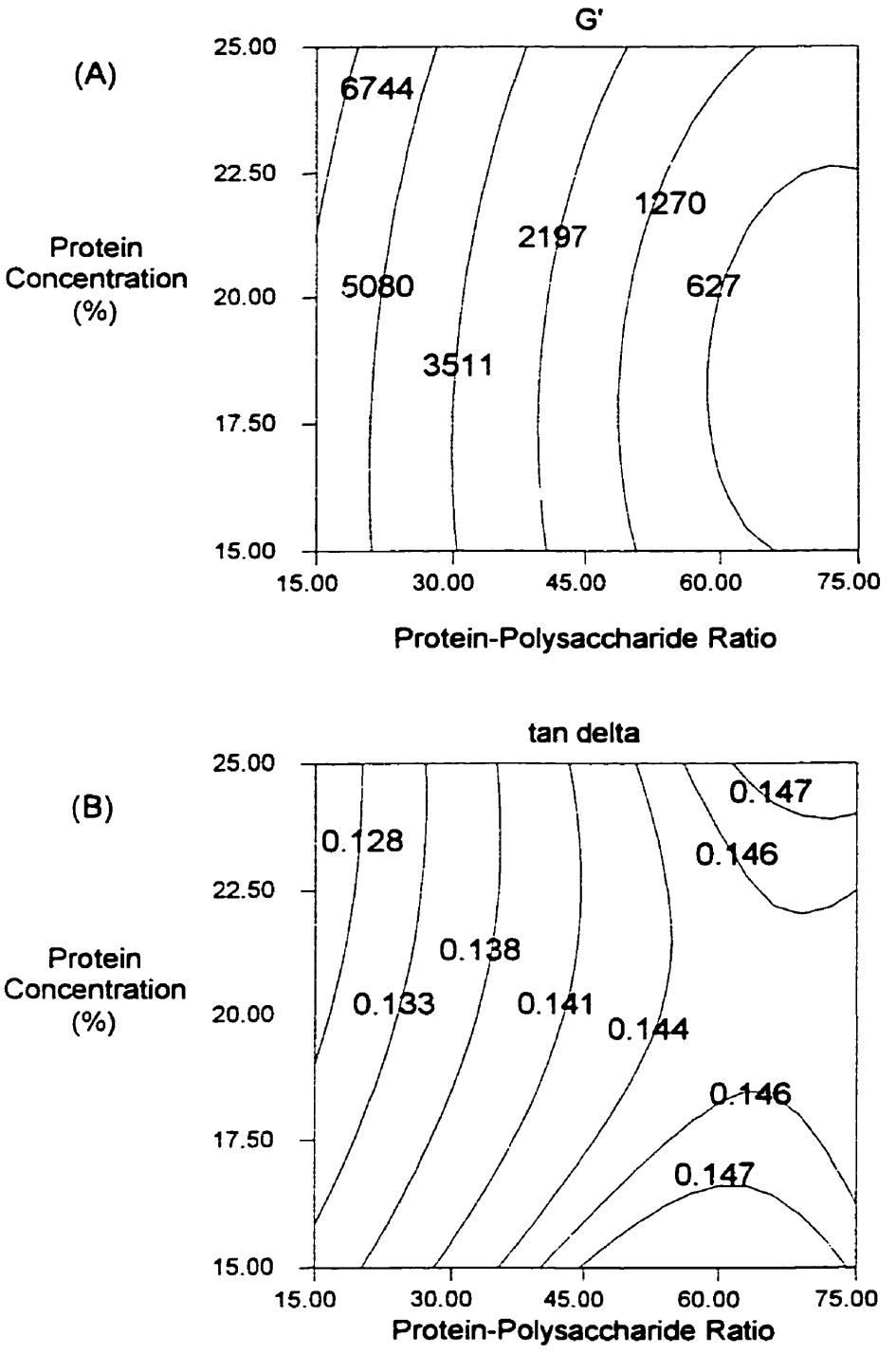
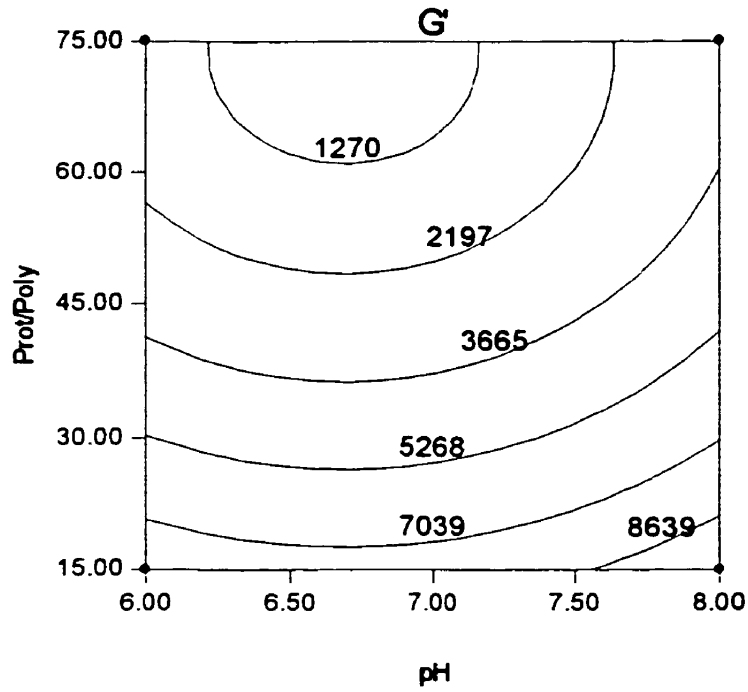



Figure 28. The effect of protein-polysaccharide ratio on both (A) storage modulus G' (Pa) (*contour lines*) and (B) loss modulus tan δ (*contour lines*) of mixed pea protein- κ-carrageenan systems at a constant pH of 7.0. (*Confirmational Optimization Experiment*)

are being used may indicate that carrageenan itself is beginning to gel and is thereby contributing to improved gelation. Nevertheless, when the factor of pH is introduced, it can be seen that rheological values become more superior as pH approaches alkalinity (Figure 29). A pH of 8 with a protein-polysaccharide ratio of 15 yielded an average tan δ value of 0.099, representative of a well formed gel. At pH 6 and the same protein to polysaccharide ratio, tan δ values rose to 0.135, indicative of a network composed of more soluble and/or aggregated material. It is known that in the case of soluble complex formation, a significant increase in gel elasticity can result (Cai 1996). In particular, Ipsen (1995) found that the highest gel strength and gel stiffness, of mixed pea protein and k-carrageenan gels, were found within the pH range of 6.4-6.8 as a result of electrostatic complexing. It was suggested that improved gelation is possible at pH 6 due to the low net charge on the protein which would still favor protein aggregation. In addition, the pH value is not so high that strong repulsive forces would be created between the highly negative protein and carrageenan molecules. However, others have demonstrated that complexing can result in a slight deterioration in gelling properties. Burova et al. (1992) found that the formation of soluble broad bean legumin-polysaccharide complexes was limited to very low protein concentrations. Furthermore, these systems resulted in colloidal rather than true solutions and consequently were unstable. Therefore, it was concluded by Burova et al. (1992) that it was difficult to prepare gels of this nature because of the low solubility of protein-polysaccharide complexes. The instability of pea protein-carrageenan complexes can further be seen in DSC and solubility testing.

(A)




(B)

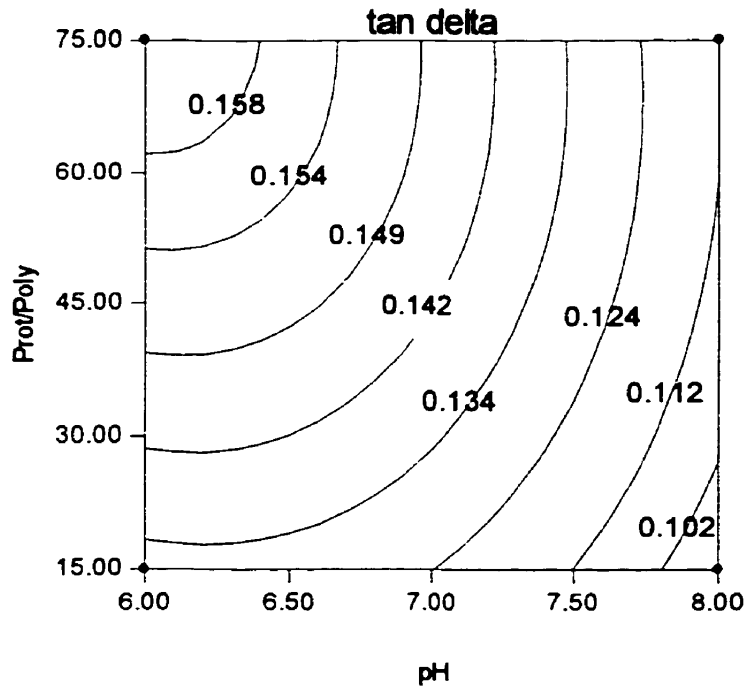



Figure 29. The interaction effect of protein-polysaccharide ratio and pH on (A) the storage modulus G' (Pa) (*contour lines*) and (B) the loss modulus tan δ (*contour lines*) of mixed systems at a constant protein concentration of 25%. (*Confirmational Optimization Experiment*)

At pH 8, pea protein has a large net negative charge, greater than that at pH 6. Due to carrageenan's negative charge it can be postulated that repulsion is occurring between the polymers. The enhanced rheological parameters seen at this pH may then be attributed to mutual exclusion. It is also apparent that between these two distinctive pHs (i.e. pH 7), the desirability of the storage modulus diminishes. According to Samant et al. (1993), the phenomenon of soluble complex formation competes with that of biopolymer incompatibility in solution. As a consequence, a combination of thermodynamic characteristics of individual polymers and of mixtures can be observed. The poor network properties could be a consequence of mixtures undergoing limited forms of both exclusion and complexing.

**2. DSC - Conformational Stability**

Because protein conformation and conformational stability can greatly influence gelling properties, pea protein-carrageenan mixtures were thermally scanned to determine possible conformational alterations caused by the inclusion of carrageenan. Responses of denaturation temperature and enthalpy can be seen in Appendices 25, 28, and 31. Initial screening of possible variables contributing to thermal parameters revealed that pH was a highly significant factor to both Td and $\Delta H$ ($p < 0.0001$) (Appendix 34 and 35). In addition, protein concentration, protein-polysaccharide ratio, and interactions between these two variables and pH were also discovered to have significant effects on Td ($p < 0.01$). It is not surprising to observe the significant effect of protein concentration, since a wide range of protein concentrations have been chosen for this experiment. A system consisting of 20% protein will increase conformational stability, as compared to 10%

protein system, due to its greater shielding effect against heat degradation. This main effect was eliminated in RSM studies as the range of protein concentration was reduced.

At an acidic pH of 4, Td and ΔH values were drastically lower than at alkaline pH values (Table 8, 9), indicating earlier unfolding and less stability within the protein system. When comparing Td values to that of guar gum systems at pH 4, temperatures were generally 2° lower. Therefore, the reduction in Td cannot be merely a function of pH. The increased presence of polysaccharide within the system (i.e. lower protein-polysaccharide ratio) was observed to cause a decline in denaturation temperatures as seen in the interaction graph (Figure 30). This influence of polysaccharide level was not as great at alkaline pHs. Based on the rheological data in this study, it was shown that mixed carrageenan systems, when in an acidic environment, resulted in the formation of insoluble complexes It would appear that the formation of insoluble complexes, due to the presence of carrageenan, may account for the lower Td values. Burova et al (1992) found that charged polysaccharides at a pH of 4.2 and at low ionic strengths decreased the thermal stability of 11s globulin from broad bean. Under conditions of incompatibility on the other hand, no effect was seen. Ledward (1994) also discovered that denaturation temperatures of broad bean legumin complexed with that of dextran and carrageenan decreased Td by as much as 12-16°C.

When the pH and polymer levels were adjusted and protein-salt ratio excluded for optimization experiments, the only main effects that were highly significant were protein-polysaccharide ratio and pH ($p < 0.0001$) (Appendix 36 and 37). Note that responses from phase one and phase two optimization experiments were combined for

TABLE 8. Effect of protein concentration, protein-polysaccharide ratio and pH on the denaturation temperature Td (°C) of mixed pea protein-carrageenan systems [a].(*Screening Experiment*)

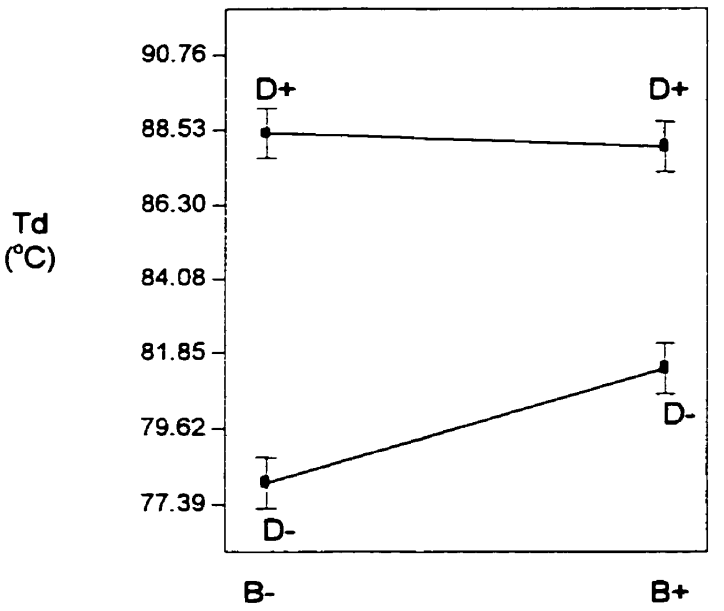
| Protein Concentration (%) | Protein/Poly Ratio | pH 4 | pH 9 |
|---|---|---|---|
| 10 | 20 | 78.23 | 86.55 |
| 20 | | 77.83 | 90.35 |
| 10 | 100 | 81.45 | 86.55 |
| 20 | | 81.33 | 89.51 |

[a] Values displayed in table are based on ranges of protein-NaCl ratios. Since protein-NaCl ratio showed no statistical significance to Td, values were consequently averaged

TABLE 9. Effect of pH on the enthalpy of denaturation ΔH (J/g) of mixed pea protein-carrageenan systems [a].(*Screening Experiment*)

| pH 4 | pH 9 |
|---|---|
| 4.95 | 12.95 |

[a] Values displayed in table are based on ranges of protein concentrations, protein-polysaccharide ratios and protein-NaCl ratios. Since these factors showed no statistical significance to ΔH, values were consequently averaged




Factors: B: Protein-Polysac. Ratio
" - " indicates ratio of 20
" +" indicates ratio of 100

D: pH
" - " indicates pH 4
" + " indicates pH 9

FIGURE 30. Interaction effect of protein-polysaccharide ratio and pH on the denaturation temperature Td (°C) of pea protein-carrageenan systems. (*Screening Experiment*)

statistical analysis. In general, Td and ΔH values declined as pH and protein-polysaccharide ratio were lowered (Figure 31). Futhermore, the interaction effect between polysaccharide level and pH continued to influene Td values ($p < 0.005$) (Appendix 36). Within the pH range used, contour graphs (Figure 31a) showed that polysaccharide levels contributed significantly to Td, but only at lower pHs. In normal circumstances, the maximum values for both Td and ΔH are expected to be around the protein's IEP, where the net surface charge is effectively neutralized. However with the inclusion of carrageenan, Td values were clearly shown to decrease as pH moved towards the IEP. It must be noted that Td values were not as low as those seen in the screening study at pH 4 where insoluble complexes developed. The decrease in conformational stability, in this pH range, may be a consequence of soluble complex formation. A decrease in the temperature of protein denaturation may be due to the unfolding of the adsorbed protein molecules surrounded by free segments of the polysaccharide chain (Tolstoguzov 1986). An increase in the dimensions of the junction zones between the polypeptides and polysaccharide chains presumably leads to a shift in the equilibrium, from native to denatured forms of the protein. Therefore a decrease in the conformational stability of the protein in complexes with anionic polysaccharides is a consequence of the preferential binding of the denatured form of the protein to the polysaccharide matrix (Burova 1992). The stability of the bound protein does not depend upon its binding density. Therefore, the preferential binding of the denatured form of the protein does not change with complexes of various compositions. Since pea protein and carrageenan are both negatively charged between 6 and 7, only weak non-cooperative complexing between the polymers will occur (Burova 1992). This compatibility between

(A)

(B)

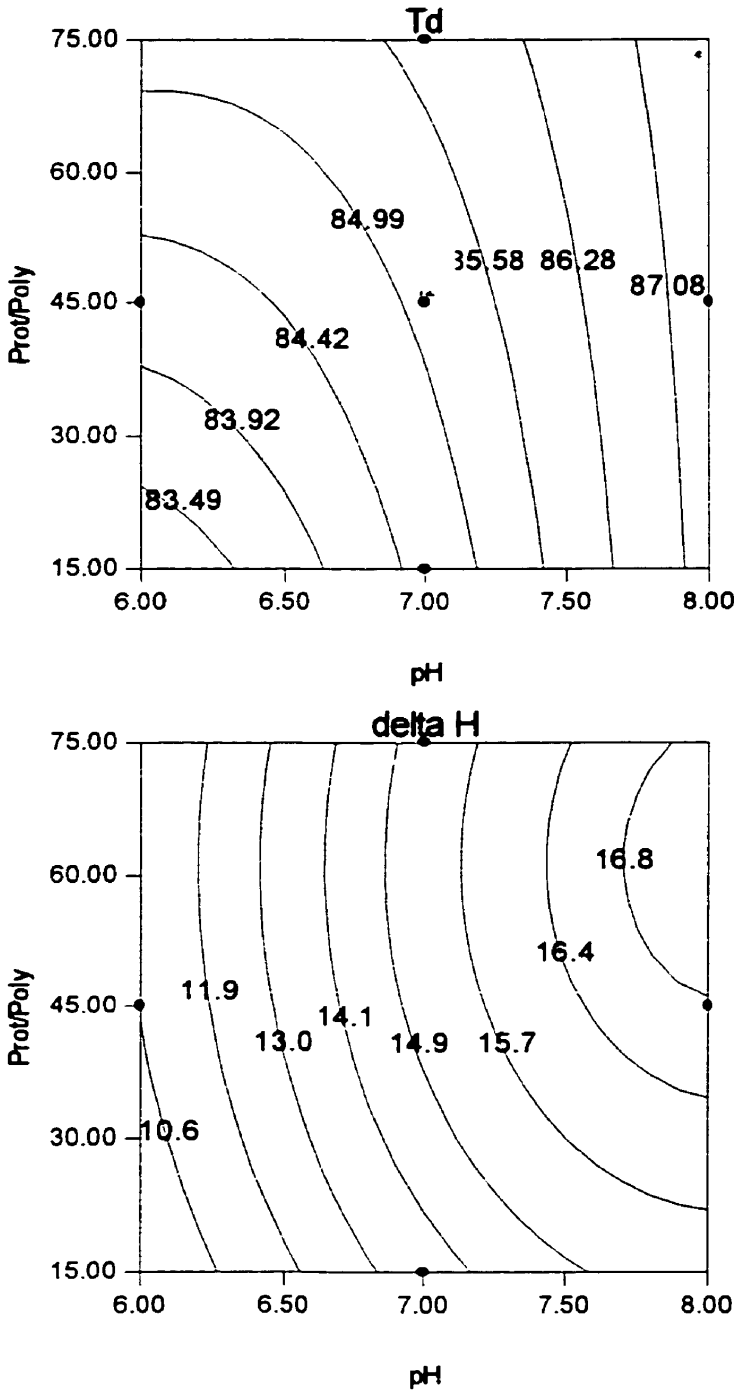



Figure 31. The effect of protein-polysaccharide ratio and pH on (A) thermal denaturation temperature Td (°C) (*contour lines*) and (B) enthalpy of denaturation ΔH (J/g) (*contour lines*) of mixed pea protein-carrageenan systems at a constant protein concentration of 20%. (*Combined Optimization Experiments*)

polymers is presumed to be the result of localized electrostatic interactions. Therefore it is likely that both complexes and free protein are present within the composite system, resulting in a denaturation temperatures lower than that of free protein. It must be noted that all the thermograms observed had single heat absorption peaks. This is contrary to findings by Burova (1992) that showed two peaks for thermograms representing mixtures of protein and sulphate-containing polysaccharides, one of which represented free protein.

At a pH of 8, carrageenan played virtually no role in altering the conformation stability of pea protein. This finding is similar to what was seen in thermodynamically incompatible guar gum systems. This supports the notion that incompatibility between pea protein and carrageenan was occurring at a pH of 8.

### 3. Solubility

Solubility responses are displayed in Appendices 25, 28, and 31. Based on the analysis of variance from the screening experiment, factors considered to be highly significant ($p < 0.005$) were protein concentration, protein-polysaccharide ratio, pH and the interaction between pH and these polymers (Appendix 38). This initial experiment eliminated protein-NaCl ratio as a significant contributing factor to protein solubility. As seen in Table 10, the level of pH primarily determined the solubility of the pea protein. In general, average solubility responses at a pH of 4 were extremely low, ranging between 0.94 to 3.49 g/100g of protein. This is due to the lack of protein-solvent interactions when near the isoelectric point. At a pH of 9, protein-solvent interactions are

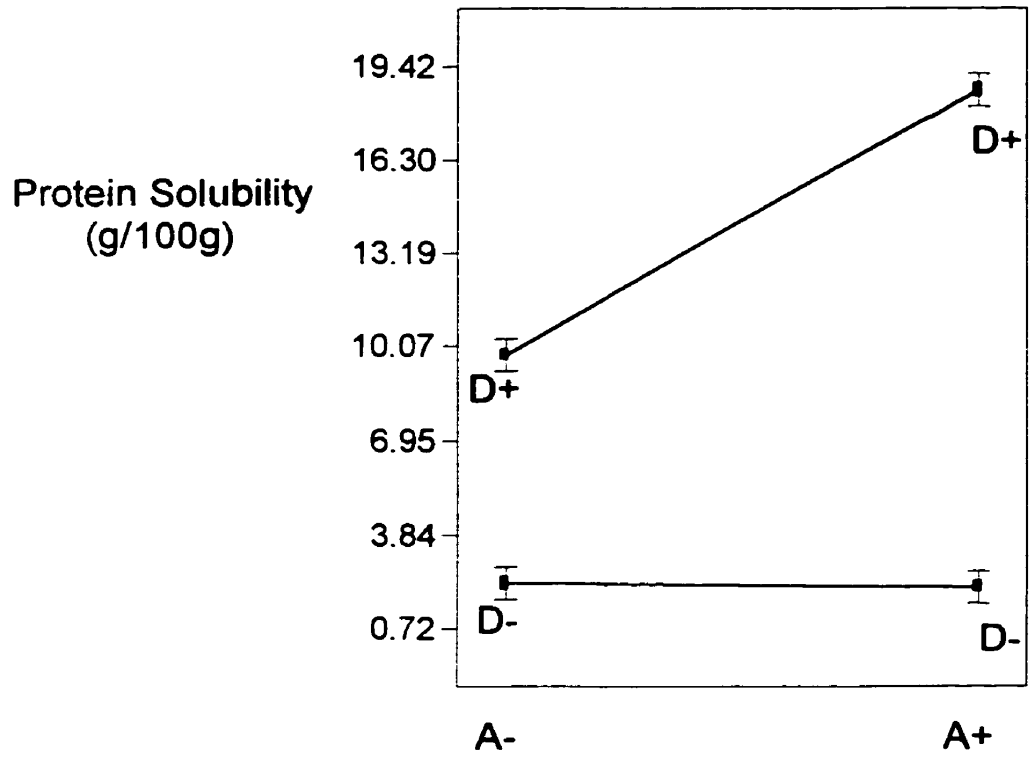
TABLE 10. Effect of protein concentration, protein-polysaccharide ratio and pH on the protein solubility (g/100g) of mixed pea protein-carrageenan systems[a]. (*Screening Experiment*)

| Protein Concentration (%) | Protein/Poly Ratio | pH 4 | pH 9 |
|---|---|---|---|
| 10 | 20 | 1.00 | 9.82 |
| 20 | | 0.94 | 18.59 |
| 10 | 100 | 3.49 | 9.74 |
| 20 | | 3.26 | 18.68 |

[a] Values displayed in table are based on ranges of protein-NaCl ratios. Since protein-NaCl ratio showed no statistical significance to protein solubility, values were consequently averaged

favored increasing solubility and allowing for the mixed system to solubilize an increasing amount of protein, with increasing protein concentration (Figure 32). On the other hand, Figure 33 illustrates that polysaccharide levels made a substantial impact only at an acidic pH. At pH 4, solubility decreased as the presence of carrageenan increased, due to the formation of insoluble complexes. During electrostatic complexing the net charge of anionic polysaccharides decreases with each successively attached protein macromolecule. This slowly reduces the net charge, hydrophilicity, and subsequent solubility of the resultant complex (Ledward 1994). The lack of influence of polysaccharide levels at pH 9 is similar to that of protein-guar gum systems. In the event that incompatibility between polymers is occurring, no effect on solubility would be seen. This is due to the fact that no change in protein conformation occurs. Rather incompatibility merely intensifies the aggregation of already denatured molecules and hence promotes protein-protein interactions.

By combining both preliminary and confirmational response surface methodology to analyze solubility responses, it was shown that protein concentration and pH were significant factors but protein-polysaccharide ratio no longer played a role in influencing solubility (Appendix 39). Solubility responses displayed in Figure 34 shows that a slight loss in solubility was experienced at pH 6. The loss is not as dramatic as that seen at a pH of 4 in the screening experiment. This may differentiate between the different types of complexing occuring within the mixed systems at these different pHs. It was the presence of polysaccharide that induced the loss in solubility at pH 4 as a result of insoluble complexing. Rheological and DSC studies have suggested, that with the inclusion of carrageenan at a pH of 6, the occurrence of soluble complex formation is




Factors: A: Protein Concentration
" - " indicates ratio of 10
" +" indicates ratio of 20

D: pH
" - " indicates pH 4
" + " indicates pH 9

FIGURE 32. Interaction effect of protein concentration and pH on the protein solubility (g/100g) of mixed pea protein-carrageenan systems.

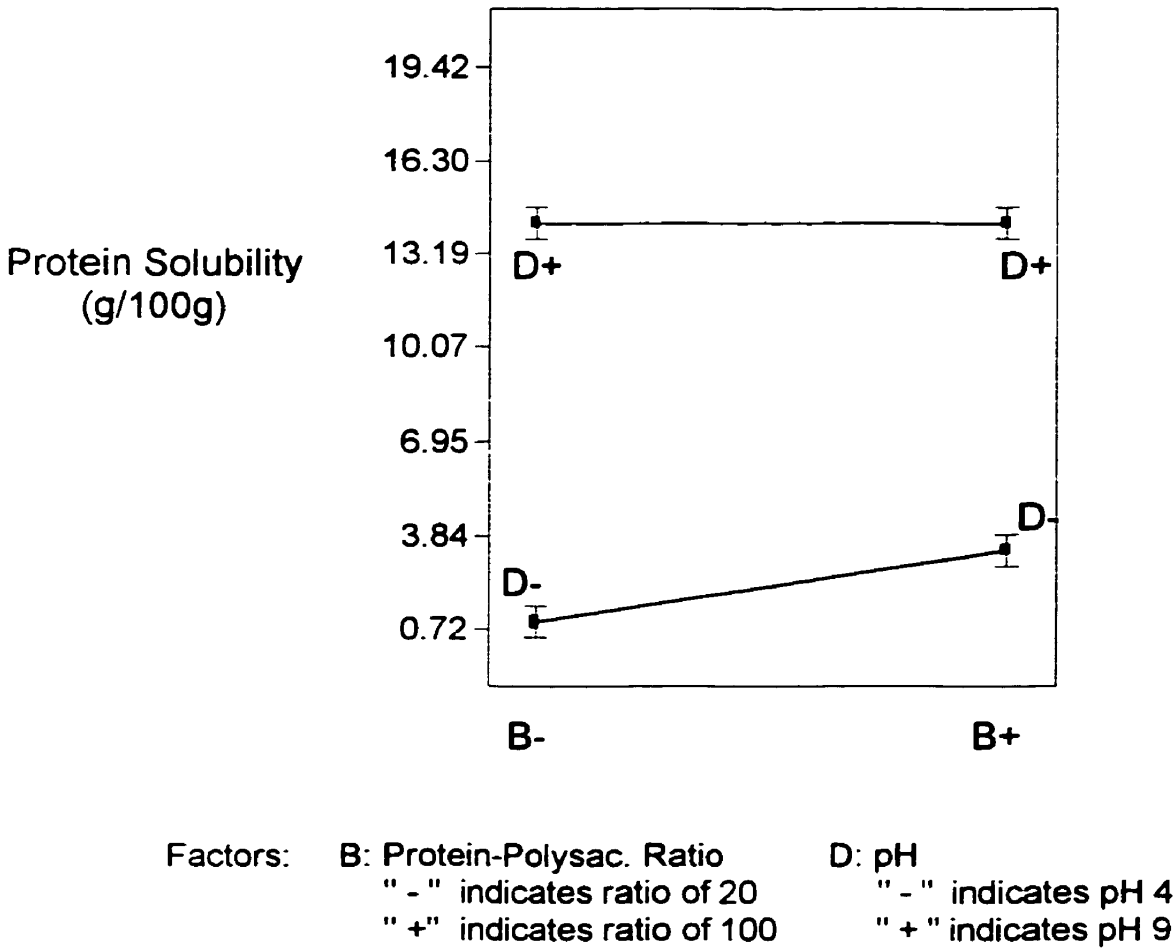



FIGURE 33. Interaction effect of protein-polysaccharide ratio and pH on the protein solubility (g/100g) of mixed pea protein-κ-carrageenan systems. (*Screening Experiment*)

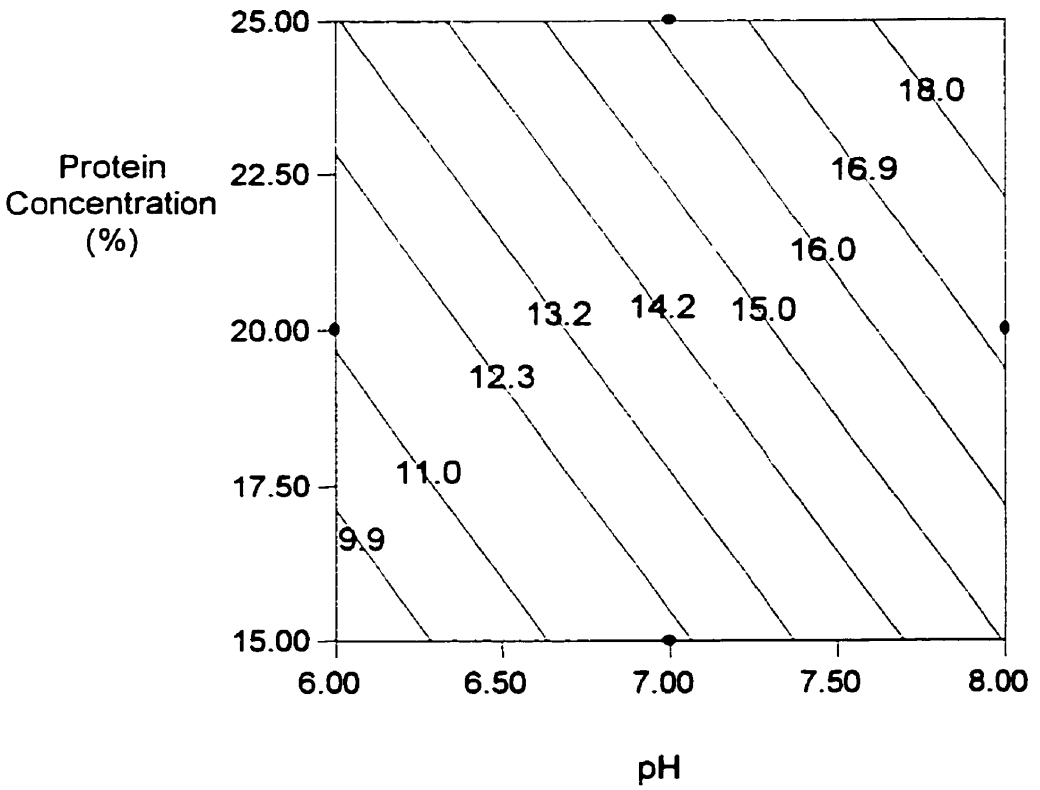



Figure 34. Effect of protein concentration (%) and pH on the solubility (g/100g) (*contour lines*) of mixed pea protein-carrageenan systems at a constant protein-polysaccharide ratio of 45. (*Combined Optimization Experiments*)

most probable. The fact that polysaccharide level is no longer significant to solubility responses, may demonstrate that while the presence of soluble complexes reduces conformational stability, it does not induce extensive conformational alteration which would cause a significant change in solubility, as in the case of insoluble complex formation. At pH 8, protein solubility remained high. For example, solubility analysis of the phase 2 experiment, detected 14.32 and 22.86 g/100g protein for mixed systems composed of 15% and 25% protein respectively. The maintenance of solubility suggests that no alteration in protein conformation took place. This in turn supports the idea that incompatibility between biopolymers is taking place at higher alkaline pHs.

**4. Numerical and Graphical Optimization**

To determine the parameters necessary for enhanced gelation of pea protein, with the inclusion of carrageenan, a simultaneous optimization of the significant factors and responses was performed. As seen previously, graphical and numerical optimizations were completed using the combination of both preliminary and confirmational response surface designs. As in the guar gum study, optimization was based on the constraints set for both factors (protein concentration, protein-polysaccharide ratio and pH) and responses (G' and tan δ). Since G' and tan δ are the central values to characterize networks, only these parameters were included in optimization procedures. Table 11 outlines the parameters set for both factors (based on alpha value = 1.6) and responses.

Based on numerical optimization, predicted solutions characterizing optimized networks is summarized in Table 11 in increasing desirability. Based on the low

| CONSTRAINTS | | | |
|---|---|---|---|
| **Name** | **Goal** | **Lower Limit** | **Upper Limit** |
| Protein Concentration (%) | w/in range | 11.59 | 28.41 |
| Prot-Polysac. Ratio | w/in range | 10.00 | 95.45 |
| pH | w/in range | 5.32 | 8.68 |
| G' (Pa) | maximize | 10000 | 20000 |
| Tan δ | target 0.05 | 0.01 | 0.11 |

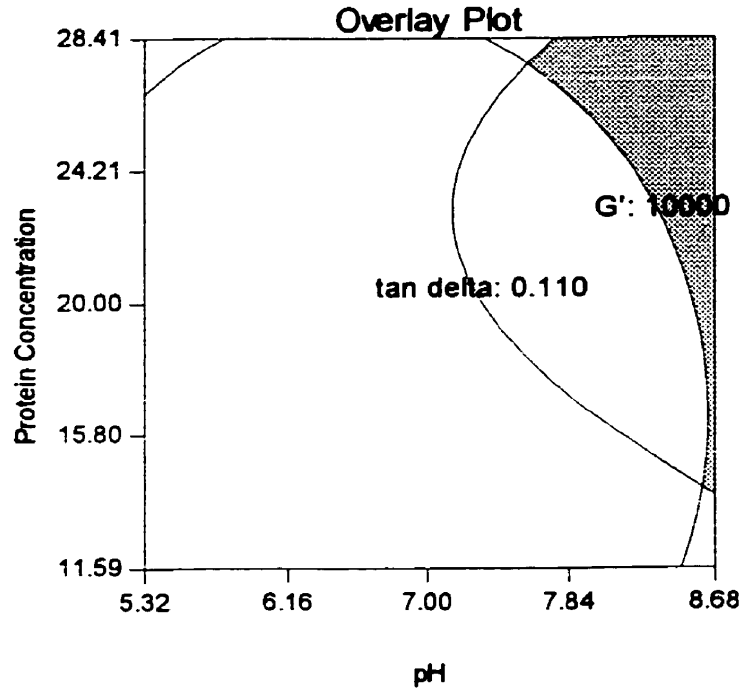
| SOLUTIONS (*Output from Numerical Optimization*) | | | | | | |
|---|---|---|---|---|---|---|
| # | Protein Conc (%) | Prot-Poly Ratio | pH | G' (Pa) | Tan δ | Desirability (%) |
| 1 | 28.41 | 10.00 | 8.68 | 14376 | 0.089 | 44.4 |
| 2 | 28.22 | 10.00 | 8.68 | 14254 | 0.088 | 44.4 |
| 3 | 28.41 | 10.52 | 8.68 | 14295 | 0.089 | 43.7 |
| 4 | 27.02 | 10.00 | 8.68 | 13471 | 0.083 | 43.0 |
| 5 | 28.41 | 10.00 | 8.30 | 12731 | 0.098 | 29.5 |
| 6 | 21.35 | 10.00 | 8.68 | 10906 | 0.076 | 23.7 |

Table 11. Predicted combinations of factors (protein concentration, protein-polysaccharide ratio, and pH) and responses (G' and tan δ) characterizing optimized protein-carrageenan networks based on set constraints within numerical optimization.

desirability levels, it is apparent that mixed protein-carrageenan systems do not form as strong a gel, within the set constraints, as compared to mixed protein-guar gum systems. However it must be noted that G' and tan δ values are still within the wide parameters set. Storage modulus values between 10,000 and 14,000 and tan delta values between 0.076 and 0.098 can still be characterized as favorable networks. As depicted by all the solutions provided, it would seem most effective to run polymer concentrations at approximately 28.41% and pH at 8.68 to maximize G' and minimize tan δ. This is somewhat opposite to guar gum systems which required low protein concentrations and a low pH. However what is similar is the low protein-polysaccharide ratio of 10.00 required to form acceptable networks.

Graphical optimization offers a more comprehensive representation of the factor and response ranges required for optimized gels since it is not limited to the level of desirability. Shaded areas within the design space indicate that networks with a G' greater than 10 000 Pa and a tan delta between 0.01 and 0.11 have been achieved. It was found that to generate a well-formed gel matrix from pea protein, with the inclusion of carrageenan, stricter and narrower parameters were required as compared to guar gum systems. In order for an improved gel matrix to be produced, pH values were required to be well above the pea protein's isoelectric point. This avoided the occurrence of insoluble complex formation, which resulted in undesirable networks. More specifically, Figure 35 showed that only pH values above approximately 7.70 were able to create desirable networks. This would suggest that the improvement of gel networks at lower alkaline pHs (i.e. 6) as a result of soluble complexing was also inadequate. Despite the

(A)




(B)

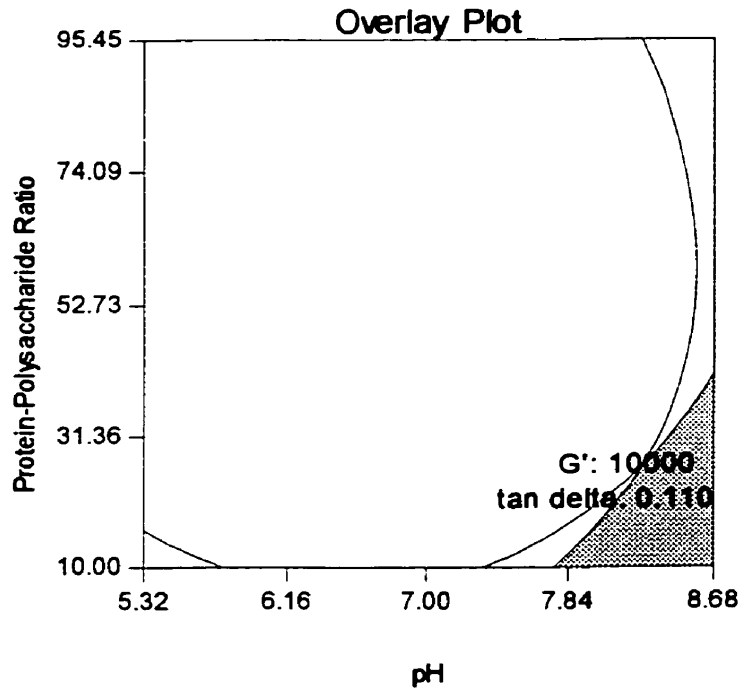



Figure 35. Graphical optimization of pea protein-carrageenan systems as a function of (A) protein concentration (%) and pH (at a constant protein-polysaccharide-ratio of 10.0) and (B) protein-polysaccharide ratio and pH (at a constant protein concentration of 28.41%).

high values of G' at both 6 and 8, as demonstrated by rheological data, tan delta values determined that more alkaline pH values were required for enhanced gelation. The improved gelation at these higher alkaline pHs was attributed to thermodynamic incompatiblity.

As pH increased from 7.70, the range of polymer levels necessary to form a gel broadened, up to a maximum level as seen at pH 8.68. Overall, the relationship between protein concentration and polysaccharide level was similar to that seen with guar gum systems. As protein concentration within a system decreased, the greater was the polysaccharide level needed to form an optimized network. At the most favorable pH (i.e. pH 8.68), protein concentration was required to be above 13.90% (Figure 35a). This points out the importance of protein levels being above a certain protein threshold within carrageenan systems. The minimal requirement of protein was not evident with the inclusion of guar gum. As pH became less desirable in carrageenan systems, the protein threshold was increased to 27.90%. The more fastidious nature of carrageenan systems was also evident by the requirement level of polysaccharide. When maintaining protein concentration and pH at the most favorable levels, protein-polysaccharide ratios could not exceed 41.30, which corresponds to polysaccharide levels being above at least 0.69%. When pH was lowered to 7.70, protein-polysaccharide ratios were required to be at a minimum level of 10.00 (approximately 2.84% polysaccharide).

# V. GENERAL DISCUSSION

The investigation into pea protein-polysaccharide systems, has revealed that two biopolymers within the same system can promote one of two phenomenon: thermodynamic incompatibility or complex formation (i.e. incompatibility). The mechanism by which gelation was encouraged and the way in which physiochemical factors influenced network formation was found to be very dependent on the thermodynamic nature of the composite mixtures. The alteration of physiochemical properties in a system induces a change in the polymer's potential within the solution. The pH level has been seen to be the most significant factor affecting the behavior of composite systems.

## A. Thermodynamically Incompatible Systems

Incompatibility between macromolecules occurs when the potential interactions between polymers are endothermic and thus are not favored (Ledward 1994). The effectiveness of a polysaccharide in contributing to incompatibility within a protein system can depend on the relative charges on the molecules, the flexibility of the chains and its molecular size (Samant et al. 1993). Guar gum exhibited incompatibility with pea protein over the entire pH range tested. This was primarily attributed to the non-ionic nature of the polysaccharide. Carrageenan, representing an anionic polysaccharide also gave way to incompatible systems but only under the higher alkaline pHs surveyed.

In order to observe improved gelation in these systems, it was necessary for polymers to repel each other to a sufficient extent to induce a concentration effect. Since the

volume of the solution, occupied by the polysaccharides, was not accessible for protein molecules the entropy of the system decreased (Ledward 1994). This in turn increased the chemical potential of pea protein. In terms of improved gelation, since the shear modulus of a gel is usually proportional to the square of its concentration (Tolstoguzov 1995), small additions of guar gum or carrageenan resulted in a several fold increase of the elastic modulus of the mixed systems.

The maintenance of a single phase system was significant in producing an improved gelled network. It must be noted that phase separation was not seen throughout this investigation. This is unusual since phase separation in protein-polysaccharide systems occur generally when the total concentration of the macromolecular components exceeds 4% (Tolstoguzov 1992). However, it also must be considered that this concentration varies for every polymer system and is influenced by various physiochemical factors. For example, according to Tolstoguzov (1998), a sulphated polysaccharide system only separates at an ionic strength above 0.5 irrespective of the pH. Phase separation has been shown to induce detrimental effects on network formation. This may have been a factor when considering the poor gelation properties of protein-guar gum systems with protein concentrations of 28.41%. Therefore in this study, it was assumed that single phase systems were maintained. As a result, thermodynamic incompatibility promoted conformational ordering within a single phase, which for gelling systems meant intensified aggregation of denatured molecules and increased rate of network formation (Samant et al 1993) .

It was concluded that pH was crucial in establishing an environment conducive to enhanced gelation. For guar gum, greater incompatibility was observed at pHs nearer to

the IEP, where the influence of charge on the protein was closer to being neutralized. For carrageenan, the incompatibility with pea protein increased as the pH was shifted away from the protein IEP. This allowed a large negative charge to develop on the pea protein inducing greater repulsive forces between the protein and the anionic polysaccharide. In both cases, solubility and thermal analysis determined that both polysaccharides had minimal effects on the conformation of pea protein suggesting the lack of interaction between polymers as would be seen in an incompatible environment

Polymer concentration also significantly contributed to improved network formation. In general, increased polymer concentration resulted in increased incompatibility as was seen by Tolstoguzov (1998). Improved networks were always observed with increased polysaccharide levels (i.e. low protein-polysaccharide ratios). In order to promote the formation of an improved network, protein levels above the critical point of gelation (>10%) were seen to be necessary. It was evident that polymer concentrations were more crucial to carrageenan than to guar gum systems. This was attributed to the fact that guar gum may be more efficient in excluding pea protein than carrageenan. According to Tolstoguzov (1995), incompatibility decreases in the order of carboxyl-containing polysaccharides > neutral polysaccharides > sulphate polysaccharides.

Conditions which promoted incompatibility between polysaccharides and pea protein resulted in the greatest improvement in gelation in this study. As eluded to previously, the inclusion of guar gum produced slightly stronger gels than that of carrageenan. For guar gum systems, numerical optimization predicted that the best gels (i.e. G' = 16 399 Pa and tan $\delta$=0.050) would be generated at a pH of 5.32 with both a low protein concentration of 11.64% and a low protein-polysaccharide ratio of 10.60. Graphical

optimization however demonstrated that optimized gels were not solely restricted to the above combination. Acceptable gels could be made within the pH range of 5.32 and 6.07. However the particular pH of the environment, strongly dictated the range of polymer levels permitted for use. Protein concentrations varying anywhere between 11.59% and 28.41% and protein-polysaccharide ratios between 10.00 and 95.45 could be used to generate a desirable gel network. It must be noted that polymer levels were influenced by how much of the other polymer was present within the system. The inclusion of carrageenan was shown to best improve pea protein gelation at a pH level of 8.68, a high protein concentration of 28.41% and a low protein-polysaccharide ratio of 10.00. This combination of parameters contributed to the formation of a gel with a G' of 14 376 Pa and a tan $\delta$ of 0.089). Graphical optimization illustrated that acceptable gels could be generated within a pH range of 7.70 and 8.68. Unlike guar gum systems however, even at the most desirable pH, polymer levels were required to be above a critical level. Enhanced gels could only be generated above a protein concentration of 13.90% and a protein-polysaccharide level below 41.30. This demonstrated the lack of flexibility in terms of physiochemical parameters in comparison to guar gum.

### B. Thermodynamically Compatible Systems

Thermodynamically compatible systems were only seen in the presence of κ-carrageenan. According to Tolstoguzov (1995), proteins and polysaccharides interact quite generally and non-specifically provided that they are close enough and can create junctions which are due solely to non-covalent bonding. Ionic bonding is one form of such interactions, although it is restricted to charged polysaccharides (Ledward 1994).

Again, pH was critical in determining the nature of the compatible system. In this study, pea protein and carrageenan were found to be compatible both above and below the protein's isoelectric point. While complexing, in both cases, was attributed to electrostatic interactions between oppositely charged polymers, there was a difference in binding at the different pHs. Basically, maximum yield of insoluble complexes corresponded to conditions of their complete mutual neutralization. Whereas soluble complexes are usually non-stoichiometric and charged. When negatively charged carrageenan molecules were added to the pea protein solution at an acidic pH, an interaction occurred, causing precipitation of reactants. A dramatic loss in solubility was observed with mixed protein-carrageenan systems as a result of insoluble complex formation. This loss in solubility was not as extreme in guar gum systems since it was presumed that incompatibility between polymers was occurring. Conformational stability was also lowered in these carrageenan systems as evidenced by lower Td values. Insoluble complex formation resulted in carrageenan systems having Td values at least 2° lower than guar gum systems in the same environment. Consequently it was observed that the presence of these insoluble complexes had a negative impact on both elasticity and network strength. The formation of large complexes are presumed to prevent unfolding of protein chains thus precluding the formation of a gel structure (Cai 1996). Therefore it can be concluded that in the case where a well developed network is desired, physiochemical conditions which promote the formation of insoluble complexes should be avoided. While these complexes do not exhibit good rheological properties, their utilization has been promoted in other applications. A number of workers have shown that it is possible to recover proteins from wastestreams and from dilute protein solutions,

by reaction with acidic polysaccharides (Tolstoguzov 1995). A method of preparing rapeseed protein isolates using acidic polymers to precipitate the proteins, after an initial alkali extraction, has also been demonstrated (Ledward 1994). A number of possible precipitants, including carrageenan, have been shown to recover protein yields of about 90%.

The method in which carrageenan is complexed to pea protein, at pHs above the IEP (i.e. pH 6), is different from what was seen below the isoelectric point. It is presumed that binding in this case was encouraged subsequent to protein unfolding and heat denaturation. While the binding mechanism is still ionic in nature, it is now due to only localized electrostatic interactions. Unlike the situation with insoluble complex formation, binding via micro-regions resulted in a complex unit which would not prevent the unfolding and entanglement of biopolymers (Cai 1996). Tolstoguzov (1992) determined that segments of polymer chains that are not incorporated into the junction zones play a key role in dictating the solubility, aggregation and gelation of the complex. Their interaction can lead to aggregation of complex particles and formation of a complex gel. The formation of soluble complexes in this study resulted in significant increases in gel elasticity (G'), comparable to that of incompatible systems but resulted in only moderately favorable loss tangent values. Correspondingly, solubility and thermal stability of pea protein was slightly diminished as compared to incompatible systems but greatly improved as compared to situations below the IEP.

# VI. CONCLUSIONS AND RECOMMENDATIONS

## A. Conclusion

The present study has shown that the inclusion of polysaccharides in pea protein systems has resulted in improved gel network formation. Enhanced gelation was not evidenced by the interaction of these polysaccharides with pea protein but rather by their incompatibility within solution. These composite gels gave much stronger gelation properties than would be anticipated from the properties of the individual components at their overall nominal concentrations. A pea protein-guar gum combination generated strengthened gels at a pH of 5.32, a low protein concentration of 11.64% and a minimal protein-polysaccharide ratio of 10.60. These gels were defined by a storage modulus of 16 399 Pa and a loss modulus of 0.050. The addition of κ-carrageenan to pea protein systems contributed to the formation of enhanced networks at a pH of 8.68, a maximum protein concentration of 28.41% and a low protein-polysaccharide ratio of 10.00. Bipolymer gels of these systems were typified by a storage modulus of 14 376 Pa and a loss modulus of 0.089.

As seen in this study it can be concluded that physiochemical parameters, depict what type of thermodynamic system will be generated between macromolecules. The nature of the polysaccharide determined to some extent its compatibility with pea protein. In particular, the non-ionic character of guar gum allowed it to be incompatible with pea protein at all pH levels studied. It was seen in this study that guar gum promoted the incompatibility between polymers to a greater extent than carrageenan and as such resulted in stronger and better gel networks. The level of pH was observed to be the most

significant factor influencing the compatibility or incompatibility of polymers. This was especially pertinent in terms of carrageenan systems. At pHs above 7.70, carrageenan was incompatible with pea protein, due to the large repulsion between the negatively charged polymers . As pH moved towards the protein's isoelectric point, interactions between the protein and polysaccharide occurred, encouraging complex formation. Rheological assessment alone could not determine the occurrence of incompatibility or compatibility. Due to a lack of interaction, incompatible systems demonstrated no effect on protein conformation and therefore no change in solubility or thermal stability. Compatible carrageenan-pea protein systems showed a decrease in solubility, Td and $\Delta H$ values. The extent to which compatibility altered conformational stability depended on the specific mechanism of interaction.

The nature of compatibility, between pea protein and carrageenan, both above and below the protein's isoelectric point differed. The opposite charges on protein and carrageenan below the IEP resulted in the association and precipiation of a large colonies in the form of insoluble complexes. At pHs above the IEP (i.e. pH 6), proteins maintaining micro-regions of net positive charge, promoted interaction with anionic carrageenan polyers resulting in the formation of soluble complexes. In compatible systems, network formation was somewhat encouraged at pH values above the IEP but suppressed at pH values below the IEP. Any improvement in the storage modulus of compatible protein-carrageenan systems (i.e. at pH 6) was offset by undesirable tan delta values and as a result such networks were incomparable with those generated from an incompatible environment.

## B. Recommendations for Further Research

1. Closer study is warranted on the significance of added guar gum to pea protein systems composed of protein concentrations below its critical gelation point. Of particular interest is the possibility of forming gels from mixed polymer solutions under conditions where the two individual polymers will not gel on their own.

2. Observation and analysis of phase separation, will allow for greater understanding and evidence of incompatible systems. This may require the examination of higher protein (i.e. above 25%) and polysaccharide levels.

3. In this study, gels were generated by heating solutions from 25-95°C followed by cooling. By expanding the heating range to over 100°C, stronger networks may be formed due to more complete unfolding of the protein.

4. Given the observations seen in this investigation, the main cause of improved gelation resulting from blending biopolymers seemed to be biopolymer incompatibility. Compatible carrageenan systems at a pH of 6 showed increased storage modulus values but undesirable tan delta values. Further study can be done on whether enhancements of gel properties in mixed polymer systems can occur by direct association of the two materials to form a coupled network. Work with divalent salts may offer some improvement with lowering tan $\delta$ values

5. The impact of polysaccharides on the rate of protein gelation, the change in the critical point of gelation and alteration during the heating and cooling cycles

should be further investigated. In this case, closer examination of mechanical spectra of these mixed gels is needed.

6. Findings in the present research was restricted to the statistical design used. Testing of additional design points, not included in the experimental design, may reveal additional insight into mechanisms of improved gelation. Different conditions may reveal the ability of locust bean gum to enhance protein gelation.

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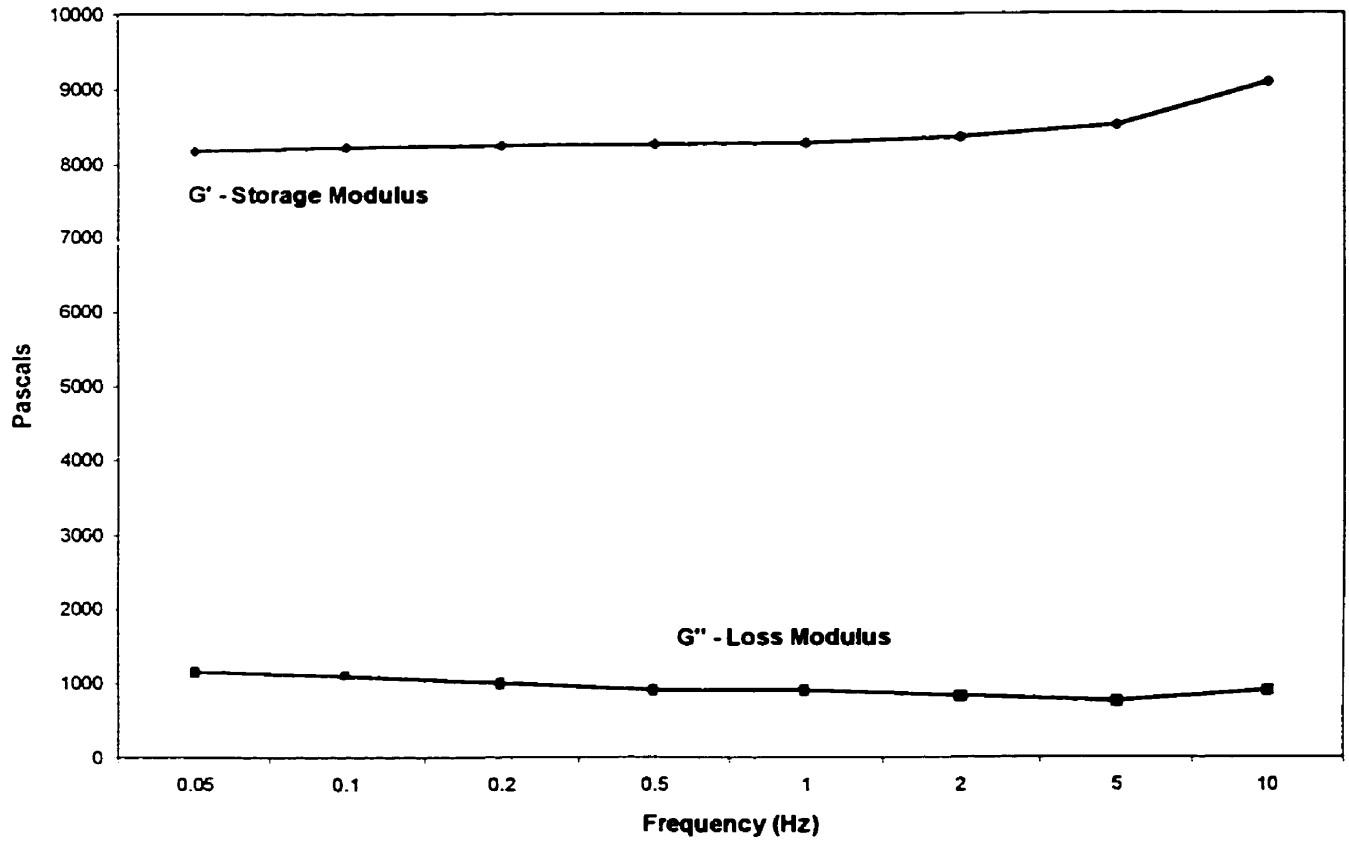
# VIII. APPENDICES




Appendix A: Typical mechanical spectra of mixed protein-polysaccharide gelled systems. (Protein Concentration = 15%, Polysaccharide Ratio=1%)

APPENDIX 1. Storage modulus G' (Pa), loss modulus tan δ, thermal denaturation temperature Td (°C), denaturation enthalpy ΔH (J/g) and solubility (g/100g) responses for mixed pea protein-locust bean gum systems.[a]

| Independent Variables | | | | Dependent Variables | | | | |
|---|---|---|---|---|---|---|---|---|
| Protein Concentration (%) | Protein/Polysac. Ratio (% Polysac.) | Protein/NaCl Ratio (% NaCl) | pH | G' (Pa) | Tan δ | ΔH J/g | Td (°C) | Solubility g/100g |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 99.40 | 0.354 | 3.30 | 97.98 | 11.83 |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 71.60 | 0.383 | 5.71 | 96.95 | 12.15 |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 110.0 | 0.284 | 3.75 | 98.54 | 11.93 |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 10.30 | 0.464 | 2.04 | 98.27 | 11.40 |
| 10 | 20 (0.50%) | 10 (1.00%) | 4.0 | 69.70 | 0.346 | 3.75 | 69.01 | 4.92 |
| 20 | 20 (1.00%) | 10 (2.00%) | 4.0 | 73.90 | 0.173 | 1.96 | 88.37 | 7.33 |
| 10 | 100 (0.10%) | 10 (1.00%) | 4.0 | 124.0 | 0.025 | 9.41 | 66.59 | 4.82 |
| 20 | 100 (0.20%) | 10 (2.00%) | 4.0 | 203.0 | 0.172 | 3.91 | 80.51 | 6.98 |
| 10 | 20 (0.50%) | 30 (0.33%) | 4.0 | 55.60 | 0.168 | 5.92 | 80.30 | 3.12 |
| 20 | 20 (1.00%) | 30 (0.67%) | 4.0 | 151.0 | 0.153 | 6.35 | 87.64 | 5.76 |
| 10 | 100 (0.10%) | 30 (0.33%) | 4.0 | 7.140 | 0.546 | 3.75 | 62.09 | 3.97 |
| 20 | 100 (0.20%) | 30 (0.67%) | 4.0 | 130.0 | 0.003 | 0.631 | 63.28 | 6.35 |
| 10 | 20 (0.50%) | 10 (1.00%) | 9.0 | 8.650 | 0.639 | 2.66 | 95.47 | 8.88 |
| 20 | 20 (1.00%) | 10 (2.00%) | 9.0 | 195.0 | 0.465 | 4.13 | 101.3 | 19.17 |
| 10 | 100 (0.10%) | 10 (1.00%) | 9.0 | 0.000 | 0.000 | 6.20 | 94.13 | 9.90 |
| 20 | 100 (0.20%) | 10 (2.00%) | 9.0 | 118.0 | 0.109 | 5.15 | 103.6 | 20.24 |
| 10 | 20 (0.50%) | 30 (0.33%) | 9.0 | 16.00 | 0.484 | 7.84 | 90.52 | 8.06 |
| 20 | 20 (1.00%) | 30 (0.67%) | 9.0 | 256.0 | 0.263 | 3.60 | 96.82 | 15.43 |
| 10 | 100 (0.10%) | 30 (0.33%) | 9.0 | 1.410 | 0.979 | 3.41 | 90.54 | 8.39 |
| 20 | 100 (0.20%) | 30 (0.67%) | 9.0 | 20.10 | 0.334 | 3.12 | 98.12 | 16.97 |

[a] Phase zero - Screening Experiment

APPENDIX 2: Screening Experiment - ANOVA of storage modulus G' of mixed pea protein-locust bean gum systems. (Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)* )

Dependent Variable: G'

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | Pa | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | Pa | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | Pa | Numeric | 10.00 | 30.00 |
| D | pH | Pa | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 46710.02 | 1 | 46710.02 | 13.34 | 0.0020 |
| Curvature | 873.18 | 1 | 873.18 | 0.25 | 0.6240 |
| Residual | 59541.64 | 17 | 3502.45 | | |
| Lack of Fit | 53542.55 | 14 | 3824.47 | 1.91 | 0.3264 |
| Pure Error | 5999.09 | 3 | 1999.70 | | |
| Cor Total | 1.071E+05 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 59.18 | R-Squared | 0.4396 | |
| Dep Mean | 86.04 | Adj R-Squared | 0.4067 | |
| C.V. | 68.78 | Pred R-Squared | 0.2476 | |
| PRESS | 80598.18 | Adeq Precision | 4.7150 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 89.34 | 1 | 14.80 | | | |
| A-Prot Conc | 54.03 | 1 | 14.80 | 3.65 | 0.0020 | 1.00 |
| Center Point | -16.52 | 1 | 33.08 | -0.50 | 0.6240 | 1.00 |

APPENDIX 3: Screening Experiment - ANOVA of loss modulus tan δ mixed pea protein-locust bean gum systems. (Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)* )

Dependent Variable: tan δ

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | | Numeric | 10.00 | 30.00 |
| D | pH | | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 0.28 | 1 | 0.28 | 5.90 | 0.0265 |
| Curvature | 0.015 | 1 | 0.015 | 0.31 | 0.5852 |
| Residual | 0.80 | 17 | 0.047 | | |
| Lack of Fit | 0.79 | 14 | 0.056 | 10.11 | 0.0407 |
| Pure Error | 0.017 | 3 | 5.50E-03 | | |
| Cor Total | 1.10 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 0.22 | R-Squared | 0.2578 | |
| Dep Mean | 0.32 | Adj R-Squared | 0.2141 | |
| C.V. | 68.78 | Pred R-Squared | 0.0363 | |
| PRESS | 1.06 | Adeq Precision | 3.137 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 0.30 | 1 | 0.054 | | | |
| BC | 0.13 | 1 | 0.054 | 2.43 | 0.0265 | 1.00 |
| Center Point | 0.068 | 1 | 0.12 | 0.56 | 0.5852 | 1.00 |

APPENDIX 4: Screening Experiment - ANOVA of temperature of denaturation Td of mixed pea protein-locust bean gum systems. (Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)* )

Dependent Variable: Td

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | °C | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | °C | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | °C | Numeric | 10.00 | 30.00 |
| D | pH | °C | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 2180.18 | 2 | 1090.09 | 26.94 | <0.0001 |
| Curvature | 493.22 | 1 | 493.22 | 12.19 | 0.0030 |
| Residual | 647.37 | 16 | 40.46 | | |
| Lack of Fit | 645.92 | 13 | 49.69 | 102.76 | 0.0014 |
| Pure Error | 1.45 | 3 | 0.48 | | |
| Cor Total | 3320.77 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 6.36 | R-Squared | 0.7711 | |
| Dep Mean | 88.00 | Adj R-Squared | 0.7424 | |
| C.V. | 7.23 | Pred R-Squared | 0.7046 | |
| PRESS | 981.01 | Adeq Precision | 10.711 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 85.52 | 1 | 1.59 | | | |
| A-Prot Conc | 4.44 | 1 | 1.59 | 2.79 | 0.0131 | 1.00 |
| D-pH | 10.80 | 1 | 1.59 | 6.79 | <0.0001 | 1.00 |
| Center Point | 12.42 | 1 | 3.56 | 3.49 | 0.6240 | 1.00 |

APPENDIX 5: Screening Experiment - ANOVA of protein solubility of mixed pea protein-locust bean gum systems. (Note: *Design Expert V5.31 only displays probability values of significant factors (p < 0.05)* )

Dependent Variable: protein solubility

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | g/100 g | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | g/100 g | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | g/100 g | Numeric | 10.00 | 30.00 |
| D | pH | g/100 g | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 445.67 | 4 | 111.42 | 215.01 | <0.0001 |
| Curvature | 18.96 | 1 | 18.96 | 36.60 | <0.0031 |
| Residual | 7.25 | 14 | 0.52 | | |
| Lack of Fit | 6.96 | 11 | 0.63 | 6.38 | 0.0768 |
| Pure Error | 0.30 | 3 | 0.099 | | |
| Cor Total | 471.88 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 0.72 | R-Squared | 0.9840 | |
| Dep Mean | 9.88 | Adj R-Squared | 0.9794 | |
| C.V. | 7.29 | Pred R-Squared | 0.9677 | |
| PRESS | 15.25 | Adeq Precision | 39.359 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 9.39 | 1 | 0.18 | | | |
| A-Prot Conc | 2.89 | 1 | 0.18 | 16.03 | <0.0001 | 1.00 |
| C-Prot/Nacl | -0.89 | 1 | 0.18 | -4.93 | 0.0002 | 1.00 |
| D-pH | 3.99 | 1 | 0.18 | 22.15 | <0.0001 | 1.00 |
| AD | 1.69 | 1 | 0.18 | 6.79 | <0.0001 | 1.00 |
| Center Point | 2.43 | 1 | 0.40 | 3.49 | <0.0001 | 1.00 |

APPENDIX 6. Storage modulus G' (Pa), loss modulus tan δ, thermal denaturation temperature Td (°C), denaturation enthalpy ΔH (J/g) and solubility (g/100g) responses for mixed pea protein-guar gum systems.[a]

| Independent Variables | | | | Dependent Variables | | | | |
|---|---|---|---|---|---|---|---|---|
| Protein Concentration (%) | Protein/Polysac. Ratio (% Polysac.) | Protein/NaCl Ratio (% NaCl) | pH | G' (Pa) | Tan δ | ΔH J/g | Td (°C) | Solubility g/100g |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 3399 | 0.114 | 8.24 | 87.07 | 12.45 |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 3415 | 0.117 | 8.61 | 87.06 | 12.60 |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 3416 | 0.116 | 7.56 | 87.08 | 13.09 |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 3420 | 0.118 | 8.79 | 87.02 | 12.17 |
| 10 | 20 (0.50%) | 10 (1.00%) | 4.0 | 203 | 0.181 | 4.40 | 83.07 | 5.43 |
| 20 | 20 (1.00%) | 10 (2.00%) | 4.0 | 989 | 0.141 | 7.51 | 84.59 | 8.32 |
| 10 | 100 (0.10%) | 10 (1.00%) | 4.0 | 129 | 0.373 | 5.05 | 82.16 | 5.33 |
| 20 | 100 (0.20%) | 10 (2.00%) | 4.0 | 656 | 0.162 | 7.92 | 84.08 | 8.06 |
| 10 | 20 (0.50%) | 30 (0.33%) | 4.0 | 191 | 0.211 | 4.05 | 82.92 | 3.94 |
| 20 | 20 (1.00%) | 30 (0.67%) | 4.0 | 743 | 0.150 | 8.10 | 83.87 | 6.98 |
| 10 | 100 (0.10%) | 30 (0.33%) | 4.0 | 66 | 0.555 | 7.93 | 84.76 | 3.50 |
| 20 | 100 (0.20%) | 30 (0.67%) | 4.0 | 595 | 0.165 | 6.53 | 83.05 | 7.18 |
| 10 | 20 (0.50%) | 10 (1.00%) | 9.0 | 443 | 0.119 | 9.67 | 88.05 | 11.28 |
| 20 | 20 (1.00%) | 10 (2.00%) | 9.0 | 7430 | 0.104 | 14.65 | 93.17 | 19.61 |
| 10 | 100 (0.10%) | 10 (1.00%) | 9.0 | 11.2 | 0.348 | 14.61 | 88.12 | 10.54 |
| 20 | 100 (0.20%) | 10 (2.00%) | 9.0 | 781 | 0.149 | 4.05 | 89.95 | 19.13 |
| 10 | 20 (0.50%) | 30 (0.33%) | 9.0 | 363 | 0.123 | 7.99 | 85.07 | 9.27 |
| 20 | 20 (1.00%) | 30 (0.67%) | 9.0 | 6264 | 0.109 | 12.85 | 90.63 | 17.25 |
| 10 | 100 (0.50%) | 30 (0.33%) | 9.0 | 4.2 | 1.254 | 7.84 | 84.16 | 9.56 |
| 20 | 100 (0.20%) | 30 (0.67%) | 9.0 | 708 | 0.156 | 10.12 | 89.44 | 17.98 |

[a] Phase zero - Screening Experiment

APPENDIX 7: Screening Experiment - ANOVA of storage modulus G' of mixed pea protein-guar gum systems. (Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)* )

Dependent Variable: G'

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | Pa | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | Pa | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | Pa | Numeric | 10.00 | 30.00 |
| D | pH | Pa | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 8420.90 | 7 | 1202.99 | 304.52 | <0.0001 |
| Curvature | 3289.74 | 1 | 3289.74 | 832.76 | <0.0001 |
| Residual | 43.45 | 11 | 3.95 | | |
| Lack of Fit | 43.44 | 8 | 5.43 | 864.15 | <0.0001 |
| Pure Error | 0.019 | 3 | 6.283E-03 | | |
| Cor Total | 11754.09 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 1.99 | R-Squared | 0.9949 | |
| Dep Mean | 32.77 | Adj R-Squared | 0.9916 | |
| C.V. | 6.07 | Pred R-Squared | 0.9852 | |
| PRESS | 173.78 | Adeq Precision | 59.982 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 26.35 | 1 | 0.50 | | | |
| A-Prot Conc | 14.72 | 1 | 0.50 | 29.63 | <0.0001 | 1.00 |
| B-Prot/Poly | -10.17 | 1 | 0.50 | 20.47 | <0.0001 | 1.00 |
| D-pH | 6.82 | 1 | 0.50 | 13.73 | <0.0001 | 1.00 |
| AB | -4.76 | 1 | 0.50 | -9.58 | <0.0001 | 1.00 |
| AD | 7.08 | 1 | 0.50 | 14.24 | <0.0001 | 1.00 |
| BD | -8.01 | 1 | 0.50 | -16.13 | <0.0001 | 1.00 |
| ABD | -4.75 | 1 | 0.50 | -9.56 | <0.0001 | 1.00 |
| Center Point | 32.06 | 1 | 1.11 | 8.86 | <0.0001 | 1.00 |

APPENDIX 8: Screening Experiment - ANOVA of loss modulus tan δ of mixed pea protein-guar gum systems. (Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)* )

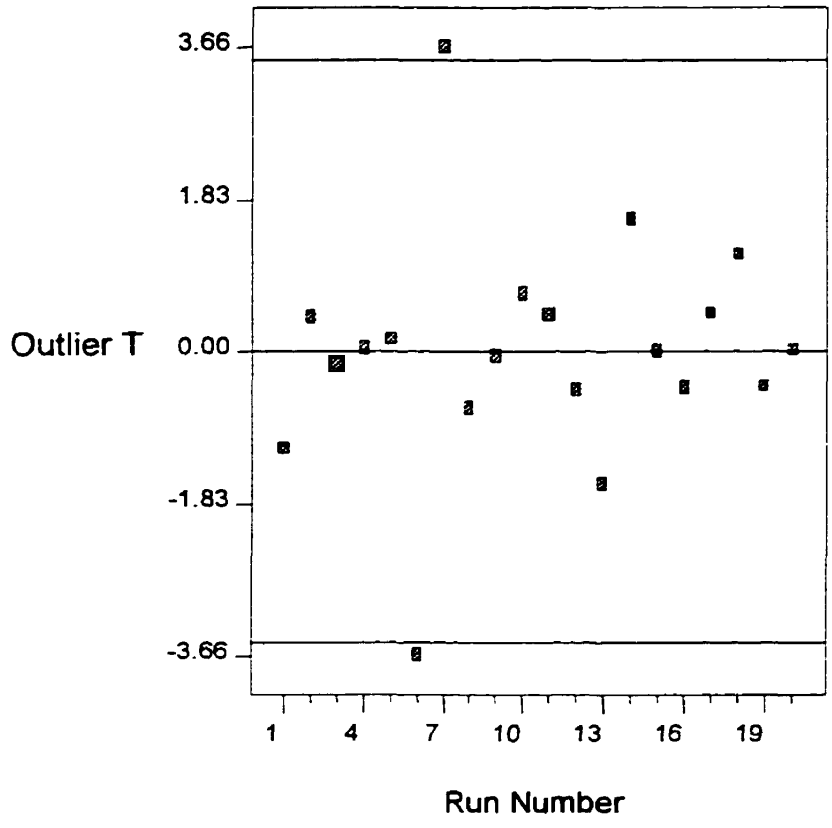
Dependent Variable: tan δ

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | | Numeric | 10.00 | 30.00 |
| D | pH | | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 0.71 | 3 | 0.24 | 6.46 | 0.0051 |
| Curvature | 0.074 | 1 | 0.074 | 2.04 | 0.1741 |
| Residual | 0.55 | 15 | 0.037 | | |
| Lack of Fit | 0.55 | 12 | 0.046 | 15667.31 | <0.0001 |
| Pure Error | 8.750E-06 | 3 | 2.917E-06 | | |
| Cor Total | 1.33 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 0.19 | R-Squared | 0.5637 | |
| Dep Mean | 0.24 | Adj R-Squared | 0.4764 | |
| C.V. | 80.25 | Pred R-Squared | 0.2677 | |
| PRESS | 0.97 | Adeq Precision | 5.4000 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 0.27 | 1 | 0.048 | | | |
| A-Prot Conc | -0.13 | 1 | 0.048 | -2.65 | 0.0181 | 1.00 |
| B-Prot/Poly | 0.13 | 1 | 0.048 | 2.65 | 0.0183 | 1.00 |
| AB | -0.11 | 1 | 0.048 | -2.31 | 0.0354 | 1.00 |
| Center Point | -0.15 | 1 | 0.110 | -1.43 | 0.1741 | 1.00 |




Appendix 9: Outlier plot of storage modulus G' (Pa) values of mixed pea protein-guar gum systems *("Run Number" corresponds to 1 of the 20 design sequences listed in Screening Experiment, Appendix 6)*

APPENDIX 10. Storage modulus G' (Pa), loss modulus tan δ, thermal denaturation temperature Td (°C), denaturation enthalpy ΔH (J/g) and solubility (g/100g) responses for mixed pea protein-guar gum systems.[a]

| Independent Variables | | | Dependent Variables | | | | |
|---|---|---|---|---|---|---|---|
| Protein Concentration (%) | Protein/Polysac. Ratio (% Polysac.) | pH | G' (Pa) | Tan δ | ΔH J/g | Td (°C) | Solubility g/100g |
| 20.00 | 45.00 (0.44%) | 7.00 | 1507 | 0.147 | 15.75 | 84.96 | 15.18 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1516 | 0.149 | 15.52 | 84.87 | 14.06 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1500 | 0.149 | 15.17 | 85.27 | 15.39 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1507 | 0.148 | 15.78 | 85.19 | 15.01 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1500 | 0.149 | 16.00 | 84.90 | 13.97 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1520 | 0.150 | 15.49 | 85.10 | 13.95 |
| 11.59 | 45.00 (0.26%) | 7.00 | 2120 | 0.120 | 16.16 | 85.00 | 10.16 |
| 28.41 | 45.00 (0.63%) | 7.00 | 790 | 0.149 | 16.23 | 86.92 | 6.35 |
| 20.00 | -5.45 (3.00%) | 7.00 | 2970 | 0.131 | 13.09 | 85.23 | 14.44 |
| 20.00 | 95.45 (0.21%) | 7.00 | 1210 | 0.142 | 16.45 | 85.33 | 13.97 |
| 20.00 | 45.00 (0.44%) | 5.32 | 10560 | 0.093 | 18.32 | 89.05 | 8.48 |
| 20.00 | 45.00 (0.44%) | 8.68 | 950 | 0.137 | 17.83 | 83.17 | 19.11 |
| 15.00 | 15.00 (1.00%) | 6.00 | 9650 | 0.109 | 14.58 | 88.33 | 10.72 |
| 25.00 | 15.00 (1.67%) | 6.00 | 9980 | 0.104 | 15.64 | 89.12 | 17.52 |
| 15.00 | 75.00 (0.20%) | 6.00 | 3450 | 0.119 | 16.13 | 88.20 | 10.93 |
| 25.00 | 75.00 (0.33%) | 6.00 | 8190 | 0.113 | 13.42 | 88.00 | 17.36 |
| 15.00 | 15.00 (1.00%) | 8.00 | 2100 | 0.127 | 16.59 | 83.69 | 13.54 |
| 25.00 | 15.00 (1.67%) | 8.00 | 2380 | 0.123 | 17.21 | 87.45 | 21.38 |
| 15.00 | 75.00 (0.20%) | 8.00 | 860 | 0.144 | 14.86 | 85.29 | 13.49 |
| 25.00 | 75.00 (0.33%) | 8.00 | 2790 | 0.119 | 16.01 | 85.86 | 20.91 |

[a] Phase one - Preliminary Optimization Experiment

APPENDIX 11: Preliminary Optimization Experiment - ANOVA of storage modulus G' (Pa) of mixed pea protein-guar gum systems.

Dependent Variable: G'

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | Pa | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | Pa | Numeric | 15.00 | 75.00 |
| C | pH | Pa | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (quad.) | 1.897E+08 | 9 | 2.108E+07 | 12.22 | 0.0003 |
| Residual | 1.726E+07 | 10 | 1.726E+06 | | |
| Lack of Fit | 1.726E+07 | 5 | 3.451E+08 | 51155.59 | <0.0001 |
| Pure Error | 337.33 | 5 | 67.47 | | |
| Cor Total | 2.070E+08 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 1313.65 | R-Squared | 0.9166 | |
| Dep Mean | 3352.50 | Adj R-Squared | 0.8416 | |
| C.V. | 39.18 | Pred R-Squared | 0.3617 | |
| PRESS | 1.321E+08 | Adeq Precision | 11.833 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 1452.52 | 1 | 535.77 | | | |
| A-Prot Conc | 369.28 | 1 | 355.47 | 1.04 | 0.3233 | 1.00 |
| B-Prot/Poly | -862.57 | 1 | 355.47 | 2.65 | 0.0357 | 1.00 |
| C-pH | -2877.82 | 1 | 355.47 | -8.10 | <0.0001 | 1.00 |
| $A^2$ | 345.89 | 1 | 346.04 | 1.00 | 0.3411 | 1.02 |
| $B^2$ | 570.39 | 1 | 346.04 | 1.65 | 0.1303 | 1.02 |
| $C^2$ | 1866.17 | 1 | 346.04 | 5.39 | 0.0003 | 1.02 |
| AB | 757.50 | 1 | 464.45 | 1.63 | 0.1339 | 1.00 |
| BC | 895.00 | 1 | 464.45 | 1.93 | 0.0828 | 1.00 |

APPENDIX 12: Preliminary Optimization Experiment - ANOVA of loss modulus tan δ of mixed pea protein-guar gum systems.

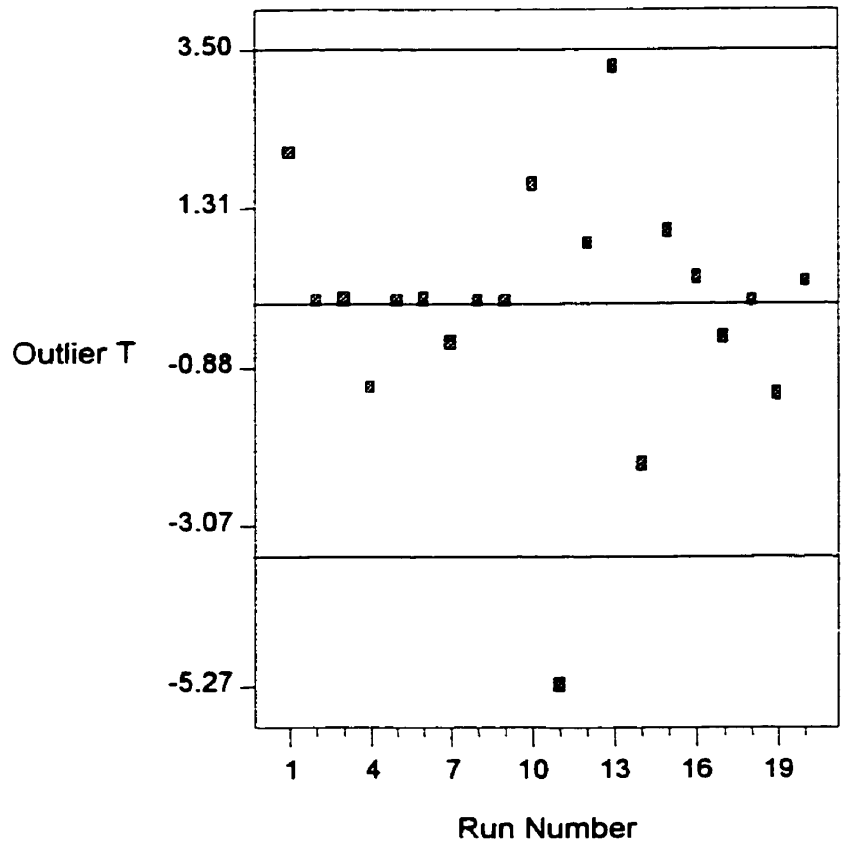
Dependent Variable: tan δ

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | | Numeric | 15.00 | 75.00 |
| C | pH | | Numeric | 6.00 | 8.00 |

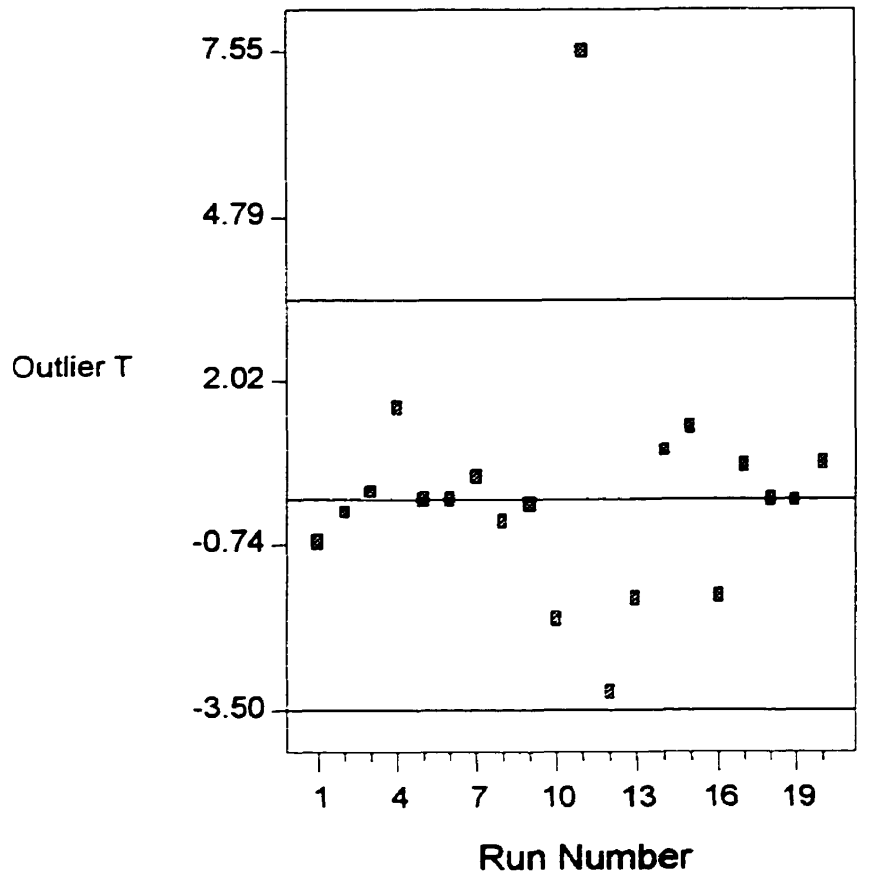
| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (quad.) | 5.067E-03 | 9 | 5.630E-04 | 6.05 | 0.0003 |
| Residual | 9.310E-04 | 10 | 9.310E-05 | | |
| Lack of Fit | 9.257E-04 | 5 | 1.851E-04 | 173.57 | <0.0001 |
| Pure Error | 5.333E-06 | 5 | 1.067E-06 | | |
| Cor Total | 5.998E-03 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 9.649E-03 | R-Squared | 0.8448 | |
| Dep Mean | 0.13 | Adj R-Squared | 0.7051 | |
| C.V. | 7.36 | Pred R-Squared | -0.1853 | |
| PRESS | 7.109E-03 | Adeq Precision | 8.1710 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 0.15 | 1 | 3.935E-03 | | | |
| A-Prot Conc | 6.423E-04 | 1 | 2.611E-03 | 0.25 | 0.8107 | 1.00 |
| B-Prot/Poly | 3.698E-03 | 1 | 2.611E-03 | 1.42 | 0.1871 | 1.00 |
| C-pH | 0.010 | 1 | 2.611E-03 | 3.98 | 0.0026 | 1.00 |
| $A^2$ | 6.634E-03 | 1 | 2.542E-03 | -2.61 | 0.0260 | 1.02 |
| $B^2$ | -5.927E-03 | 1 | 2.542E-03 | -2.33 | 0.0419 | 1.02 |
| $C^2$ | -0.014 | 1 | 2.542E-03 | -5.32 | 0.0003 | 1.02 |
| AB | -2.750E-03 | 1 | 3.411E-03 | -0.81 | 0.4389 | 1.00 |
| AC | -2.250E-03 | 1 | 3.411E-03 | -0.66 | 0.5244 | 1.00 |
| BC | -7.500E-04 | 1 | 3.411E-03 | -0.22 | 0.8304 | 1.00 |




Appendix 13: Outlier plot of storage modulus G' (Pa) values of mixed pea protein-guar gum systems (*"Run Number" corresponds to 1 of the 20 design sequences listed in Preliminary Optimization Experiment, Appendix 5)*




Appendix 14: Outlier plot of loss modulus tan δ values of mixed pea protein-guar gum systems *("Run Number" corresponds to 1 of the 20 design sequences listed in Preliminary Optimization Experiment, Appendix 10)*

APPENDIX 15. Storage modulus G' (Pa), loss modulus tan δ, thermal denaturation temperature Td (°C), denaturation enthalpy ΔH (J/g) and solubility (g/100g) responses for mixed pea protein-guar gum systems.[a]

| Independent Variables | | | Dependent Variables | | | | |
|---|---|---|---|---|---|---|---|
| Protein Concentration (%) | Protein/Polysac. Ratio (% Polysac.) | pH | G' (Pa) | Tan δ | ΔH J/g | Td (°C) | Solubility g/100g |
| 20.00 | 45.00 (0.44%) | 7.00 | 1560 | 0.156 | 14.94 | 86.14 | 14.29 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1580 | 0.149 | 15.13 | 86.28 | 14.87 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1540 | 0.152 | 15.02 | 86.19 | 16.02 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1540 | 0.153 | 14.98 | 86.34 | 15.64 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1580 | 0.151 | 15.10 | 86.22 | 14.88 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1530 | 0.149 | 14.96 | 86.19 | 15.06 |
| 15.00 | 45.00 (0.33%) | 7.00 | 3420 | 0.111 | 19.33 | 85.67 | 14.28 |
| 25.00 | 45.00 (0.55%) | 7.00 | 3430 | 0.130 | 17.51 | 87.92 | 18.34 |
| 20.00 | 15.00 (1.33%) | 7.00 | 4100 | 0.131 | 14.69 | 86.18 | 14.66 |
| 20.00 | 75.00 (0.27%) | 7.00 | 1950 | 0.123 | 16.34 | 86.62 | 14.59 |
| 20.00 | 45.00 (0.44%) | 6.00 | 8960 | 0.110 | 7.85 | 85.67 | 14.92 |
| 20.00 | 45.00 (0.44%) | 8.00 | 2060 | 0.128 | 16.51 | 86.43 | 19.58 |
| 15.00 | 15.00 (1.00%) | 6.00 | 9950 | 0.109 | 15.21 | 85.35 | 10.83 |
| 25.00 | 15.00 (1.67%) | 6.00 | 10520 | 0.098 | 18.39 | 87.37 | 18.16 |
| 15.00 | 75.00 (0.20%) | 6.00 | 5100 | 0.115 | 16.09 | 85.05 | 11.25 |
| 25.00 | 75.00 (0.33%) | 6.00 | 8350 | 0.108 | 14.63 | 86.99 | 17.54 |
| 15.00 | 15.00 (1.00%) | 8.00 | 1740 | 0.123 | 13.93 | 88.52 | 14.00 |
| 25.00 | 15.00 (1.67%) | 8.00 | 2340 | 0.132 | 12.97 | 90.29 | 22.18 |
| 15.00 | 75.00 (0.20%) | 8.00 | 920 | 0.138 | 16.24 | 88.77 | 14.25 |
| 25.00 | 75.00 (0.33%) | 8.00 | 2350 | 0.115 | 14.01 | 92.42 | 22.45 |

[a] Phase two - Confirmational Optimization Experiment

APPENDIX 16: Confirmational Optimization Experiment - ANOVA of storage modulus G' (Pa) of mixed pea protein-guar gum systems.

Dependent Variable: G'

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | Pa | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | Pa | Numeric | 15.00 | 75.00 |
| C | pH | Pa | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (quad.) | 1.785E+08 | 9 | 1.983E+07 | 27.31 | <0.0001 |
| Residual | 7.261E+06 | 10 | 7.261E+05 | | |
| Lack of Fit | 7.259E+06 | 5 | 1.452E+06 | 3088.79 | <0.0001 |
| Pure Error | 2350.00 | 5 | 470.00 | | |
| Cor Total | 1.857E+08 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 852.12 | R-Squared | 0.9609 | |
| Dep Mean | 3726.00 | Adj R-Squared | 0.9257 | |
| C.V. | 22.87 | Pred R-Squared | 0.7755 | |
| PRESS | 4.170E+07 | Adeq Precision | 16.367 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 2091.91 | 1 | 292.94 | | | |
| A-Prot Conc | 586.00 | 1 | 269.46 | 0.25 | 0.0547 | 1.00 |
| B-Prot/Poly | -998.00 | 1 | 269.46 | 1.42 | 0.0041 | 1.00 |
| C-pH | -3347.00 | 1 | 269.46 | 3.98 | <0.0001 | 1.00 |
| $A^2$ | 527.73 | 1 | 513.84 | -2.61 | 0.3286 | 1.02 |
| $B^2$ | 127.73 | 1 | 513.84 | -2.33 | 0.8087 | 1.02 |
| $C^2$ | 2612.73 | 1 | 513.84 | -5.32 | 0.0005 | 1.02 |
| AB | 438.75 | 1 | 301.27 | -0.81 | 0.1760 | 1.00 |
| AC | 223.75 | 1 | 301.27 | -0.66 | 0.4748 | 1.00 |
| BC | 776.25 | 1 | 301.27 | -0.22 | 0.0276 | 1.00 |

APPENDIX 17: Confirmational Optimization Experiment - ANOVA of loss modulus tan δ of mixed pea protein-guar gum systems.

Dependent Variable: tan δ

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | | Numeric | 15.00 | 75.00 |
| C | pH | | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (quad.) | 4.346E-03 | 9 | 4.829E-04 | 2.65 | 0.0722 |
| Residual | 1.819E-03 | 10 | 1.819E-04 | | |
| Lack of Fit | 1.784E-03 | 5 | 3.568E-04 | 50.48 | 0.0003 |
| Pure Error | 3.433E-05 | 5 | 7.067E-06 | | |
| Cor Total | 6.165E-03 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 0.013 | R-Squared | 0.7049 | |
| Dep Mean | 0.13 | Adj R-Squared | 0.4394 | |
| C.V. | 0.45 | Pred R-Squared | -0.9210 | |
| PRESS | 0.012 | Adeq Precision | 4.5770 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 0.14 | 1 | 4.637E-03 | | | |
| A-Prot Conc | -1.300E-03 | 1 | 4.265E-03 | -0.30 | 0.8107 | 1.00 |
| B-Prot/Poly | 6.000E-04 | 1 | 4.265E-03 | 0.14 | 0.1871 | 1.00 |
| C-pH | 9.600E-03 | 1 | 4.265E-03 | 2.25 | 0.0026 | 1.00 |
| $A^2$ | 0.011 | 1 | 8.133E-03 | -1.41 | 0.0260 | 1.02 |
| $B^2$ | -5.000E-03 | 1 | 8.133E-03 | -0.61 | 0.0419 | 1.02 |
| $C^2$ | -0.013 | 1 | 8.133E-03 | -1.60 | 0.0003 | 1.02 |
| AB | -3.500E-03 | 1 | 4.769E-03 | -0.73 | 0.4389 | 1.00 |
| AC | -5.000E-04 | 1 | 4.769E-03 | -0.10 | 0.5244 | 1.00 |
| BC | -2.250E-04 | 1 | 4.769E-03 | -0.47 | 0.8304 | 1.00 |

APPENDIX 18: Screening Experiment - ANOVA of thermal denaturation temperature Td (°C) of mixed pea protein-guar gum systems.
(Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)* )

Dependent Variable: Td

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | °C | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | °C | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | °C | Numeric | 10.00 | 30.00 |
| D | pH | °C | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 126.64 | 2 | 63.32 | 25.46 | <0.0001 |
| Curvature | 3.13 | 1 | 3.13 | 1.26 | 0.2783 |
| Residual | 39.79 | 16 | 2.49 | | |
| Lack of Fit | 39.78 | 13 | 3.06 | 4424.56 | <0.0001 |
| Pure Error | 2.075E-03 | 3 | 6.917E-04 | | |
| Cor Total | 169.56 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 1.58 | R-Squared | 0.7609 | |
| Dep Mean | 86.27 | Adj R-Squared | 0.7311 | |
| C.V. | 1.83 | Pred R-Squared | 0.6446 | |
| PRESS | 60.27 | Adeq Precision | 10.734 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 86.07 | 1 | 0.39 | | | |
| A-Prot Conc | 1.28 | 1 | 0.39 | 3.25 | 0.0051 | 1.00 |
| D-pH | 2.51 | 1 | 0.39 | 6.36 | <0.0001 | 1.00 |
| Center Point | 0.99 | 1 | 0.88 | 1.12 | 0.2783 | 1.00 |

APPENDIX 19: Screening Experiment - ANOVA of enthalpy of denaturation ΔH (J/g) of mixed pea protein-guar gum systems. (Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)* )

Dependent Variable: ΔH

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | J/g | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | J/g | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | J/g | Numeric | 10.00 | 30.00 |
| D | pH | J/g | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 57.25 | 1 | 57.25 | 8.39 | 0.0100 |
| Curvature | 2.430E-03 | 1 | 2.430E-03 | 3.561E-04 | 0.9852 |
| Residual | 116.01 | 17 | 6.82 | | |
| Lack of Fit | 115.12 | 14 | 8.22 | 27.84 | 0.0095 |
| Pure Error | 0.89 | 3 | 0.30 | | |
| Cor Total | 173.26 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 2.61 | R-Squared | 0.3304 | |
| Dep Mean | 8.32 | Adj R-Squared | 0.2910 | |
| C.V. | 31.38 | Pred R-Squared | 0.1231 | |
| PRESS | 151.94 | Adeq Precision | 3.7390 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 8.33 | 1 | 0.65 | | | |
| D-pH | 1.89 | 1 | 0.65 | 2.90 | 0.0100 | 1.00 |
| Center Point | -0.028 | 1 | 0.65 | -0.019 | 0.9852 | 1.00 |

APPENDIX 20: Combined Optimization Experiment - ANOVA of thermal denaturation temperature Td (°C) of mixed pea protein-guar gum systems.

Dependent Variable: Td

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | °C | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | °C | Numeric | 15.00 | 75.00 |
| C | pH | °C | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (linear) | 17.75 | 3 | 5.92 | 1.92 | 0.1441 |
| Residual | 111.05 | 36 | 3.08 | | |
| Lack of Fit | 52.01 | 17 | 3.06 | 0.98 | 0.5094 |
| Pure Error | 59.04 | 19 | 3.11 | | |
| Cor Total | 128.80 | 39 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 1.76 | R-Squared | 0.1378 | |
| Dep Mean | 86.54 | Adj R-Squared | 0.0660 | |
| C.V. | 2.03 | Pred R-Squared | -0.1553 | |
| PRESS | 148.80 | Adeq Precision | 5.0630 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 86.54 | 1 | 0.28 | | | |
| A-Prot Conc | 0.84 | 1 | 0.36 | 2.32 | 0.0264 | 1.00 |
| B-Prot/Poly | 0.045 | 1 | 0.36 | 0.13 | 0.9012 | 1.00 |
| C-pH | -0.22 | 1 | 0.36 | 0.61 | 0.5428 | 1.00 |

APPENDIX 21: Combined Optimization Experiment - ANOVA of denaturation enthalpy ΔH (J/g) of mixed pea protein-guar gum systems.

Dependent Variable: ΔH

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | J/g | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | J/g | Numeric | 15.00 | 75.00 |
| C | pH | J/g | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (linear) | 2.44 | 3 | 0.81 | 0.22 | 0.8803 |
| Residual | 131.76 | 36 | 3.66 | | |
| Lack of Fit | 110.05 | 17 | 6.47 | 5.67 | 0.0002 |
| Pure Error | 21.71 | 19 | 1.14 | | |
| Cor Total | 134.20 | 39 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 1.91 | R-Squared | 0.0182 | |
| Dep Mean | 15.50 | Adj R-Squared | -0.0636 | |
| C.V. | 12.34 | Pred R-Squared | -0.2224 | |
| PRESS | 164.04 | Adeq Precision | 1.7880 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 15.50 | 1 | 0.30 | | | |
| A-Prot Conc | -0.13 | 1 | 0.39 | -0.33 | 0.7448 | 1.00 |
| B-Prot/Poly | 0.18 | 1 | 0.39 | 0.45 | 0.6567 | 1.00 |
| C-pH | 0.24 | 1 | 0.39 | 0.60 | 0.5531 | 1.00 |

APPENDIX 22: Screening Experiment - ANOVA of protein solubility of mixed pea protein-guar gum systems. (Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)* )

Dependent Variable: protein solubility

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | g/100 g | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | g/100 g | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | g/100 g | Numeric | 10.00 | 30.00 |
| D | pH | g/100 g | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 438.13 | 4 | 109.53 | 994.97 | <0.0001 |
| Curvature | 17.94 | 1 | 17.94 | 162.93 | <0.0031 |
| Residual | 1.54 | 14 | 0.11 | | |
| Lack of Fit | 1.10 | 11 | 0.10 | 0.67 | 0.7289 |
| Pure Error | 0.45 | 3 | 0.15 | | |
| Cor Total | 457.61 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 0.33 | R-Squared | 0.9965 | |
| Dep Mean | 10.68 | Adj R-Squared | 0.9955 | |
| C.V. | 3.11 | Pred R-Squared | 0.9932 | |
| PRESS | 3.11 | Adeq Precision | 85.002 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_o$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 10.21 | 1 | 0.083 | | | |
| A-Prot Conc | 2.85 | 1 | 0.083 | 34.40 | <0.0001 | 1.00 |
| C-Prot/Nacl | -0.75 | 1 | 0.083 | -9.07 | <0.0001 | 1.00 |
| D-pH | 4.12 | 1 | 0.083 | 49.64 | <0.0001 | 1.00 |
| Center Point | 2.37 | 1 | 0.190 | 12.76 | <0.0001 | 1.00 |

APPENDIX 23: Combined Optimization Experiment - ANOVA of protein solubility (g/100 g) of mixed pea protein-guar gum systems.

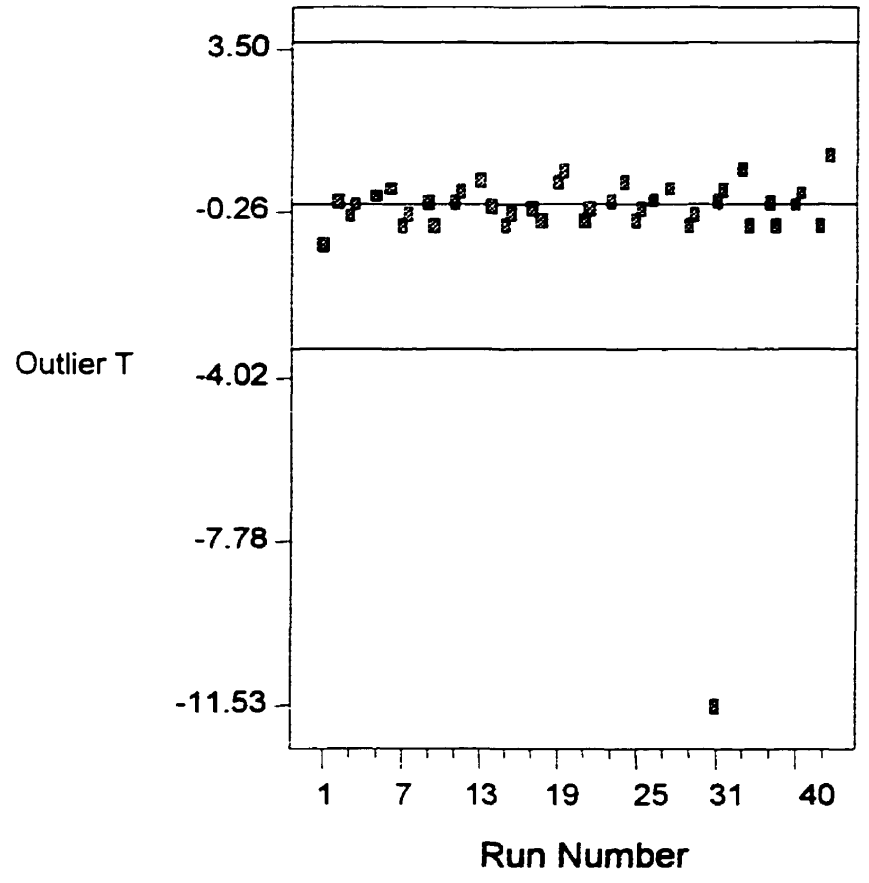
Dependent Variable: protein solubility

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | g/100 g | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | g/100 g | Numeric | 15.00 | 75.00 |
| C | pH | g/100 g | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (linear) | 240.77 | 3 | 80.26 | 12.07 | <0.0001 |
| Residual | 239.42 | 36 | 6.65 | | |
| Lack of Fit | 268.66 | 17 | 13.66 | 36.23 | <0.0001 |
| Pure Error | 7.16 | 19 | 0.38 | | |
| Cor Total | 480.20 | 39 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 2.58 | R-Squared | 0.5014 | |
| Dep Mean | 15.09 | Adj R-Squared | 0.4599 | |
| C.V. | 17.09 | Pred R-Squared | 0.3259 | |
| PRESS | 323.68 | Adeq Precision | 11.153 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 15.09 | 1 | 0.41 | | | |
| A-Prot Conc | 2.37 | 1 | 0.53 | 4.48 | <0.0001 | 1.00 |
| B-Prot/Poly | -0.043 | 1 | 0.53 | -0.081 | 0.9362 | 1.00 |
| C-pH | 2.13 | 1 | 0.53 | 4.02 | 0.0003 | 1.00 |




Appendix 24: Outlier plot of loss modulus tan δ values of mixed pea protein-guar gum systems (*"Run Number 31 corresponds to design sequence 8 listed in Preliminary Optimization Experiment, Appendix 10)*

APPENDIX 25. Storage modulus G' (Pa), loss modulus tan δ, thermal denaturation temperature Td (°C), denaturation enthalpy ΔH (J/g) and solubility (g/100g) responses for mixed pea protein-κ-carrageenan systems.[a]

| Independent Variables | | | | Dependent Variables | | | | |
|---|---|---|---|---|---|---|---|---|
| Protein Concentration (%) | Protein/Polysac. Ratio (% Polysac.) | Protein/NaCl Ratio (% NaCl) | pH | G' (Pa) | Tan δ | ΔH J/g | Td (°C) | Solubility g/100g |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 2740 | 0.180 | 7.84 | 85.79 | 7.42 |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 2460 | 0.175 | 7.61 | 85.62 | 7.21 |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 2590 | 0.176 | 7.56 | 85.47 | 7.93 |
| 15 | 60 (0.25%) | 20 (0.75%) | 6.5 | 2280 | 0.171 | 7.79 | 85.63 | 7.65 |
| 10 | 20 (0.50%) | 10 (1.00%) | 4.0 | 134 | 0.312 | 4.40 | 78.41 | 1.07 |
| 20 | 20 (1.00%) | 10 (2.00%) | 4.0 | 357 | 0.309 | 5.05 | 78.26 | 0.72 |
| 10 | 100 (0.10%) | 10 (1.00%) | 4.0 | 87.7 | 0.555 | 3.51 | 81.87 | 3.98 |
| 20 | 100 (0.20%) | 10 (2.00%) | 4.0 | 184 | 0.215 | 4.85 | 81.67 | 2.69 |
| 10 | 20 (0.50%) | 30 (0.33%) | 4.0 | 68.9 | 0.450 | 4.70 | 78.04 | 0.92 |
| 20 | 20 (1.00%) | 30 (0.67%) | 4.0 | 319 | 0.314 | 4.92 | 77.39 | 1.15 |
| 10 | 100 (0.10%) | 30 (0.33%) | 4.0 | 56.2 | 0.589 | 6.03 | 81.02 | 2.99 |
| 20 | 100 (0.20%) | 30 (0.67%) | 4.0 | 179 | 0.235 | 6.15 | 80.98 | 3.82 |
| 10 | 20 (0.50%) | 10 (1.00%) | 9.0 | 466 | 0.166 | 13.66 | 88.10 | 11.13 |
| 20 | 20 (1.00%) | 10 (2.00%) | 9.0 | 4750 | 0.127 | 13.89 | 90.76 | 18.97 |
| 10 | 100 (0.10%) | 10 (1.00%) | 9.0 | 1.164 | 0.733 | 14.61 | 88.24 | 9.85 |
| 20 | 100 (0.20%) | 10 (2.00%) | 9.0 | 743 | 0.191 | 14.04 | 89.48 | 19.42 |
| 10 | 20 (0.50%) | 30 (0.33%) | 9.0 | 225 | 0.185 | 13.46 | 85.00 | 8.50 |
| 20 | 20 (1.00%) | 30 (0.67%) | 9.0 | 2590 | 0.132 | 10.92 | 89.93 | 18.21 |
| 10 | 100 (0.10%) | 30 (0.33%) | 9.0 | 9.41 | 0.901 | 12.86 | 84.85 | 9.63 |
| 20 | 100 (0.20%) | 30 (0.67%) | 9.0 | 695 | 0.189 | 10.12 | 89.54 | 17.93 |

[a] Phase zero - Screening Experiment

APPENDIX 26: Screening Experiment - ANOVA of storage modulus G' of mixed pea protein-κ-carrageenan. (Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)* )

Dependent Variable: G'

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | Pa | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | Pa | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | Pa | Numeric | 10.00 | 30.00 |
| D | pH | Pa | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 1.533E+07 | 4 | 3.833E+06 | 6.46 | 0.0037 |
| Curvature | 1.082E+07 | 1 | 1.082E+07 | 18.23 | 0.0008 |
| Residual | 8.308E+06 | 14 | 5.934E+05 | | |
| Lack of Fit | 8.193E+06 | 11 | 7.448E+05 | 19.52 | 0.0161 |
| Pure Error | 1.145E+05 | 3 | 3.8158.33 | | |
| Cor Total | 3.445E+07 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 770.32 | R-Squared | 0.6486 | |
| Dep Mean | 1046.77 | Adj R-Squared | 0.5481 | |
| C.V. | 73.59 | Pred R-Squared | 0.4910 | |
| PRESS | 1.754E+07 | Adeq Precision | 7.0560 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 679.09 | 1 | 192.58 | | | |
| A-Prot Conc | 548.04 | 1 | 192.58 | 2.85 | 0.0130 | 1.00 |
| B-Prot/Poly | -434.65 | 1 | 192.58 | -2.26 | 0.0405 | 1.00 |
| D-pH | 505.86 | 1 | 192.58 | 2.63 | 0.0199 | 1.00 |
| AD | 461.51 | 1 | 192.58 | 2.40 | 0.0311 | 1.00 |
| Center Point | 1838.41 | 1 | 430.62 | 4.27 | 0.0008 | 1.00 |

APPENDIX 27: Screening Experiment - ANOVA of storage modulus tan δ of mixed pea protein-κ-carrageenan systems. (Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)* )

Dependent Variable: tan δ

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | | Numeric | 10.00 | 30.00 |
| D | pH | | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 337.13 | 5 | 67.43 | 70.81 | <0.0001 |
| Curvature | 8.78 | 1 | 8.78 | 9.22 | 0.0095 |
| Residual | 12.38 | 13 | 0.95 | | |
| Lack of Fit | 12.33 | 10 | 1.23 | 72.12 | 0.0024 |
| Pure Error | 0.051 | 3 | 0.017 | | |
| Cor Total | 358.29 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 0.98 | R-Squared | 0.9646 | |
| Dep Mean | 84.30 | Adj R-Squared | 0.9510 | |
| C.V. | 1.16 | Pred R-Squared | 0.9117 | |
| PRESS | 31.65 | Adeq Precision | 21.207 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 83.97 | 1 | 0.24 | | | |
| A-Prot Conc | 0.78 | 1 | 0.24 | 3.20 | 0.0070 | 1.00 |
| B-Prot/Poly | 0.73 | 1 | 0.24 | 3.01 | 0.0100 | 1.00 |
| D-pH | 4.27 | 1 | 0.24 | 17.49 | <0.0001 | 1.00 |
| AB | 0.91 | 1 | 0.24 | 3.73 | 0.0025 | 1.00 |
| BD | -0.94 | 1 | 0.24 | -3.87 | 0.0019 | 1.00 |
| Center Point | -1.66 | 1 | 0.55 | 3.04 | 0.0095 | 1.00 |

APPENDIX 28. Storage modulus G' (Pa), loss modulus tan δ, thermal denaturation temperature Td (°C), denaturation enthalpy ΔH (J/g) and solubility (g/100g) responses for mixed pea protein-κ-carrageenan systems.[a]

| Independent Variables | | | Dependent Variables | | | | |
|---|---|---|---|---|---|---|---|
| Protein Concentration (%) | Protein/Polysac. Ratio (% Polysac.) | pH | G' (Pa) | Tan δ | ΔH J/g | Td (°C) | Solubility g/100g |
| 20.00 | 45.00 (0.44%) | 7.00 | 1690 | 0.135 | 15.86 | 84.96 | 15.11 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1700 | 0.138 | 14.63 | 85.13 | 14.82 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1680 | 0.132 | 15.25 | 85.09 | 15.54 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1680 | 0.132 | 16.00 | 85.07 | 15.43 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1630 | 0.139 | 16.21 | 84.92 | 14.97 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1710 | 0.129 | 15.75 | 85.10 | 15.36 |
| 11.59 | 45.00 (0.26%) | 7.00 | 241 | 0.251 | 13.21 | 84.03 | 9.62 |
| 28.41 | 45.00 (0.63%) | 7.00 | 3590 | 0.141 | 12.44 | 83.95 | 18.07 |
| 20.00 | -5.45 (3.00%) | 7.00 | 3190 | 0.121 | 10.39 | 83.94 | 7.64 |
| 20.00 | 95.45 (0.21%) | 7.00 | 1430 | 0.163 | 16.50 | 86.49 | 12.98 |
| 20.00 | 45.00 (0.44%) | 5.32 | 960 | 0.213 | 7.55 | 83.87 | 6.15 |
| 20.00 | 45.00 (0.44%) | 8.68 | 4810 | 0.112 | 16.02 | 89.46 | 20.10 |
| 15.00 | 15.00 (1.00%) | 6.00 | 7120 | 0.152 | 8.19 | 83.19 | 9.70 |
| 25.00 | 15.00 (1.67%) | 6.00 | 8160 | 0.141 | 8.04 | 83.08 | 17.46 |
| 15.00 | 75.00 (0.20%) | 6.00 | 982 | 0.184 | 8.91 | 84.84 | 10.04 |
| 25.00 | 75.00 (0.33%) | 6.00 | 1100 | 0.171 | 8.76 | 84.66 | 20.65 |
| 15.00 | 15.00 (1.00%) | 8.00 | 8280 | 0.109 | 14.75 | 86.41 | 14.23 |
| 25.00 | 15.00 (1.67%) | 8.00 | 9990 | 0.099 | 15.40 | 87.22 | 23.62 |
| 15.00 | 75.00 (0.20%) | 8.00 | 2570 | 0.114 | 15.01 | 86.37 | 14.41 |
| 25.00 | 75.00 (0.33%) | 8.00 | 2960 | 0.111 | 16.32 | 87.20 | 22.09 |

[a] Phase one - Preliminary Optimization Experiment

APPENDIX 29: Preliminary Optimization Experiment - ANOVA of storage modulus G' (Pa) of mixed pea protein-κ-carrageenan systems.

Dependent Variable: G'

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | | Numeric | 15.00 | 75.00 |
| C | pH | | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (linear) | 7.915E+07 | 3 | 2.638E+07 | 5.56 | 0.0083 |
| Residual | 7.590E+07 | 16 | 4.743E+06 | | |
| Lack of Fit | 7.589E+07 | 11 | 6.899E+06 | 8883.12 | <0.0001 |
| Pure Error | 3883.33 | 5 | 776.67 | | |
| Cor Total | 1.550E+08 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 2177.95 | R-Squared | 0.5105 | |
| Dep Mean | 3273.65 | Adj R-Squared | 0.4187 | |
| C.V. | 66.53 | Pred R-Squared | 0.1660 | |
| PRESS | 1.293E+08 | Adeq Precision | 7.6230 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 3273.65 | 1 | 487.00 | | | |
| A-Prot Conc | 650.98 | 1 | 589.35 | 1.10 | 0.2857 | 1.00 |
| B-Prot/Poly | -2116.00 | 1 | 589.35 | -3.59 | 0.0024 | 1.00 |
| C-pH | 945.53 | 1 | 589.35 | 1.60 | 0.1282 | 1.00 |

APPENDIX 30: Preliminary Optimization Experiment - ANOVA of loss modulus tan δ of mixed pea protein-κ-carrageenan systems.

Dependent Variable: tan δ

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | | Numeric | 15.00 | 75.00 |
| C | pH | | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (linear) | 0.016 | 3 | 5.365E-03 | 8.31 | 0.0015 |
| Residual | 0.010 | 16 | 6.458E-04 | | |
| Lack of Fit | 0.010 | 11 | 9.325E-04 | 62.31 | 0.0001 |
| Pure Error | 7.483E-05 | 5 | 1.497E-05 | | |
| Cor Total | 0.026 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 0.025 | R-Squared | 0.6090 | |
| Dep Mean | 0.14 | Adj R-Squared | 0.5357 | |
| C.V. | 17.60 | Pred R-Squared | 0.3052 | |
| PRESS | 0.018 | Adeq Precision | 9.7480 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | V!F |
|---|---|---|---|---|---|---|
| Intercept | 0.14 | 1 | 5.682E-03 | | | |
| A-Prot Conc | -0.016 | 1 | 6.877E-03 | -2.36 | 0.0311 | 1.00 |
| B-Prot/Poly | 0.011 | 1 | 6.877E-03 | 1.59 | 0.1306 | 1.00 |
| C-pH | -0.028 | 1 | 6.877E-03 | -4.10 | 0.0008 | 1.00 |

APPENDIX 31. Storage modulus G' (Pa), loss modulus tan δ, thermal denaturation temperature Td (°C), denaturation enthalpy ΔH (J/g) and solubility (g/100g) responses for mixed pea protein-κ-carrageenan systems.[a]

| Independent Variables | | | Dependent Variables | | | | |
|---|---|---|---|---|---|---|---|
| Protein Concentration (%) | Protein/Polysac. Ratio (% Polysac.) | pH | G' (Pa) | Tan δ | ΔH J/g | Td (°C) | Solubility G/100g |
| 20.00 | 45.00 (0.44%) | 7.00 | 1620 | 0.141 | 15.34 | 85.16 | 15.34 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1650 | 0.145 | 15.29 | 85.21 | 15.29 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1640 | 0.144 | 15.28 | 85.28 | 15.28 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1590 | 0.140 | 15.46 | 85.15 | 15.46 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1630 | 0.142 | 15.22 | 85.32 | 15.22 |
| 20.00 | 45.00 (0.44%) | 7.00 | 1610 | 0.139 | 15.37 | 85.14 | 15.37 |
| 15.00 | 45.00 (0.33%) | 7.00 | 1570 | 0.149 | 14.01 | 84.17 | 14.01 |
| 25.00 | 45.00 (0.55%) | 7.00 | 3120 | 0.141 | 13.15 | 83.46 | 13.15 |
| 20.00 | 15.00 (1.33%) | 7.00 | 5890 | 0.129 | 10.42 | 83.34 | 10.42 |
| 20.00 | 75.00 (0.27%) | 7.00 | 1290 | 0.144 | 16.34 | 86.56 | 16.34 |
| 20.00 | 45.00 (0.44%) | 6.00 | 2190 | 0.149 | 8.52 | 83.69 | 8.52 |
| 20.00 | 45.00 (0.44%) | 8.00 | 4290 | 0.113 | 15.41 | 90.01 | 15.41 |
| 15.00 | 15.00 (1.00%) | 6.00 | 7400 | 0.139 | 9.16 | 83.24 | 9.16 |
| 25.00 | 15.00 (1.67%) | 6.00 | 8350 | 0.131 | 9.04 | 83.04 | 9.04 |
| 15.00 | 75.00 (0.20%) | 6.00 | 1210 | 0.156 | 9.29 | 84.80 | 9.29 |
| 25.00 | 75.00 (0.33%) | 6.00 | 1480 | 0.161 | 8.76 | 84.73 | 8.76 |
| 15.00 | 15.00 (1.00%) | 8.00 | 8410 | 0.105 | 15.13 | 86.52 | 15.13 |
| 25.00 | 15.00 (1.67%) | 8.00 | 10050 | 0.094 | 14.99 | 87.66 | 14.99 |
| 15.00 | 75.00 (0.20%) | 8.00 | 2610 | 0.113 | 15.06 | 86.52 | 15.06 |
| 25.00 | 75.00 (0.33%) | 8.00 | 2870 | 0.111 | 15.48 | 87.59 | 15.48 |

[a] Phase two - Confirmational Optimization Experiment

APPENDIX 32: Confirmational Optimization Experiment - ANOVA of storage modulus G' (Pa) of mixed pea protein-κ-carrageenan systems.

Dependent Variable: G'

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | | Numeric | 15.00 | 75.00 |
| C | pH | | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (quad.) | 1.520E+08 | 9 | 1.689E+07 | 78.80 | <0.0001 |
| Residual | 2.144E+06 | 10 | 2.144E+05 | | |
| Lack of Fit | 2.142E+06 | 5 | 4.283E+05 | 917.81 | <0.0001 |
| Pure Error | 2333.33 | 5 | 466.67 | | |
| Cor Total | 1.542E+08 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 463.02 | R-Squared | 0.9861 | |
| Dep Mean | 3523.50 | Adj R-Squared | 0.9736 | |
| C.V. | 13.14 | Pred R-Squared | 0.8900 | |
| PRESS | 1.696E+07 | Adeq Precision | 29.211 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 1715.09 | 1 | 159.18 | | | |
| A-Prot Conc | 467.00 | 1 | 146.42 | 3.19 | 0.0107 | 1.00 |
| B-Prot/Poly | -3064.00 | 1 | 146.42 | -20.93 | <0.0001 | 1.00 |
| C-pH | 760.00 | 1 | 146.42 | 5.19 | 0.0004 | 1.00 |
| $A^2$ | 492.27 | 1 | 279.21 | 1.76 | 0.1084 | 1.82 |
| $B^2$ | 1737.27 | 1 | 279.21 | 6.22 | <0.0001 | 1.82 |
| $C^2$ | 1387.27 | 1 | 279.21 | 4.97 | 0.0006 | 1.82 |
| AB | -257.50 | 1 | 163.70 | -1.57 | 0.1468 | 1.00 |
| AC | 85.00 | 1 | 163.70 | 0.52 | 0.6149 | 1.00 |
| BC | 10.00 | 1 | 163.70 | 0.061 | 0.9525 | 1.00 |

APPENDIX 33: Confirmational Optimization Experiment - ANOVA of loss modulus tan δ of mixed pea protein-κ-carrageenan systems.

Dependent Variable: tan δ

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | | Numeric | 15.00 | 75.00 |
| C | pH | | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (quad.) | 6.019E-03 | 9 | 6.688E-04 | 130.96 | <0.0001 |
| Residual | 5.107E-05 | 10 | 5.107E-06 | | |
| Lack of Fit | 2.424E-05 | 5 | 4.847E-06 | 0.90 | 0.5431 |
| Pure Error | 2.683E-05 | 5 | 5.367E-06 | | |
| Cor Total | 6.071E-03 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 2.260E-03 | R-Squared | 0.9916 | |
| Dep Mean | 0.13 | Adj R-Squared | 0.9840 | |
| C.V. | 1.68 | Pred R-Squared | 0.9620 | |
| PRESS | 2.308E-04 | Adeq Precision | 40.927 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 0.14 | 1 | 7.769E-04 | | | |
| A-Prot Conc | -0.016 | 1 | 7.146E-04 | -3.22 | 0.0192 | 1.00 |
| B-Prot/Poly | 0.011 | 1 | 7.146E-04 | 12.31 | <0.0001 | 1.00 |
| C-pH | -0.028 | 1 | 7.146E-04 | -27.85 | <0.0001 | 1.00 |
| $A^2$ | 2.273E-03 | 1 | 1.363E-03 | 1.67 | 0.1263 | 1.00 |
| $B^2$ | -6.227E-03 | 1 | 1.363E-03 | -4.57 | 0.0010 | 1.00 |
| $C^2$ | -0.012 | 1 | 1.363E-03 | 8.61 | <0.0001 | 1.00 |
| AB | 2.875E-03 | 1 | 7.990E-04 | 3.60 | 0.0049 | 1.00 |
| AC | -1.125E-03 | 1 | 7.990E-04 | -1.41 | 0.1894 | 1.00 |
| BC | -2.625E-03 | 1 | 7.990E-04 | -3.29 | 0.0182 | 1.00 |

APPENDIX 34: Screening Experiment - ANOVA of denaturation temperature Td (°C) of mixed pea protein-κ-carrageenan systems. (Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)*)

Dependent Variable: Td

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | °C | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | °C | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | °C | Numeric | 10.00 | 30.00 |
| D | pH | °C | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 337.13 | 5 | 67.43 | 70.81 | <0.0001 |
| Curvature | 8.78 | 1 | 8.78 | 9.22 | 0.0095 |
| Residual | 12.38 | 13 | 0.95 | | |
| Lack of Fit | 12.33 | 10 | 1.23 | 72.12 | 0.0024 |
| Pure Error | 0.051 | 3 | 0.017 | | |
| Cor Total | 358.29 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 0.98 | R-Squared | 0.9646 | |
| Dep Mean | 84.30 | Adj R-Squared | 0.9510 | |
| C.V. | 1.16 | Pred R-Squared | 0.9117 | |
| PRESS | 31.65 | Adeq Precision | 21.207 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 83.97 | 1 | 0.24 | | | |
| A-Protein Con. | 0.78 | 1 | 0.24 | 3.20 | 0.0070 | 1.00 |
| B-Prot/Poly | 0.73 | 1 | 0.24 | 3.01 | 0.0100 | 1.00 |
| D-pH | 4.27 | 1 | 0.24 | 17.49 | <0.0001 | 1.00 |
| AD | 0.91 | 1 | 0.24 | 3.73 | 0.0025 | 1.00 |
| BD | -0.94 | 1 | 0.24 | -3.87 | 0.0019 | 1.00 |
| Center Point | 1.66 | 1 | 0.55 | 3.04 | 0.0095 | 1.00 |

APPENDIX 35: Screening Experiment - ANOVA of enthalpy of denaturation ΔH (J/g) (°C) of mixed pea protein-κ-carrageenan systems. (Note: *Design Expert V5.31 only displays probability values of significant factors ($p < 0.05$)* )

Dependent Variable: ΔH

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | J/g | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | J/g | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | J/g | Numeric | 10.00 | 30.00 |
| D | pH | J/g | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 255.67 | 1 | 255.67 | 190.38 | <0.0001 |
| Curvature | 4.97 | 1 | 4.97 | 3.70 | 0.0712 |
| Residual | 22.83 | 17 | 1.34 | | |
| Lack of Fit | 22.78 | 14 | 1.63 | 90.49 | 0.0017 |
| Pure Error | 0.054 | 3 | 0.018 | | |
| Cor Total | 283.48 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 1.16 | R-Squared | 0.9180 | |
| Dep Mean | 8.70 | Adj R-Squared | 0.9132 | |
| C.V. | 13.32 | Pred R-Squared | 0.8947 | |
| PRESS | 29.84 | Adeq Precision | 17.813 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 8.95 | 1 | 0.29 | | | |
| D-pH | 4.00 | 1 | 0.29 | 13.80 | <0.0001 | 1.00 |
| Center Point | 1.66 | 1 | 0.65 | -1.92 | 0.0712 | 1.00 |

APPENDIX 36: Preliminary Optimization Experiment - ANOVA of thermal denaturation temperature Td (°C) of mixed pea protein-κ-carrageenan systems.

Dependent Variable: Td

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | °C | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | °C | Numeric | 15.00 | 75.00 |
| C | pH | °C | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (quad.) | 45.31 | 9 | 5.03 | 63.91 | <0.0001 |
| Residual | 0.79 | 10 | 0.079 | | |
| Lack of Fit | 0.75 | 5 | 0.15 | 21.04 | 0.0023 |
| Pure Error | 0.036 | 5 | 7.150E-03 | | |
| Cor Total | 46.10 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 0.28 | R-Squared | 0.9829 | |
| Dep Mean | 85.25 | Adj R-Squared | 0.9675 | |
| C.V. | 0.33 | Pred R-Squared | 0.8749 | |
| PRESS | 5.77 | Adeq Precision | 33.012 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 85.04 | 1 | 0.11 | | | |
| A-Prot Conc | 0.089 | 1 | 0.076 | 1.17 | 0.2684 | 1.00 |
| B-Prot/Poly | 0.55 | 1 | 0.076 | 7.19 | <0.0001 | 1.00 |
| C-pH | 1.53 | 1 | 0.076 | 20.08 | <0.0001 | 1.00 |
| $A^2$ | -0.36 | 1 | 0.074 | -4.86 | 0.0007 | 1.02 |
| $B^2$ | 0.074 | 1 | 0.074 | 1.00 | 0.3398 | 1.02 |
| $C^2$ | 0.59 | 1 | 0.074 | 7.94 | <0.0001 | 1.02 |
| AB | -6.250E-03 | 1 | 0.099 | -0.063 | 0.9510 | 1.00 |
| AC | 0.24 | 1 | 0.099 | 2.43 | 0.0354 | 1.00 |
| BC | -0.41 | 1 | 0.099 | -4.14 | 0.0020 | 1.00 |

APPENDIX 37: Preliminary Optimization Experiment - ANOVA of enthalpy of denaturation ΔH (J/g) of mixed pea protein-guar gum systems.

Dependent Variable: ΔH

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | J/g | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | J/g | Numeric | 15.00 | 75.00 |
| C | pH | J/g | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (quad.) | 192.32 | 9 | 21.37 | 15.63 | <0.0001 |
| Residual | 13.67 | 10 | 1.37 | | |
| Lack of Fit | 11.99 | 5 | 2.40 | 7.12 | 0.0252 |
| Pure Error | 1.68 | 5 | 0.34 | | |
| Cor Total | 205.99 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 1.17 | R-Squared | 0.9336 | |
| Dep Mean | 13.26 | Adj R-Squared | 0.8739 | |
| C.V. | 8.82 | Pred R-Squared | 0.5460 | |
| PRESS | 93.51 | Adeq Precision | 12.460 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_o$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 15.64 | 1 | 0.48 | | | |
| A-Prot Conc | 0.027 | 1 | 0.32 | 0.084 | 0.9343 | 1.00 |
| B-Prot/Poly | 0.94 | 1 | 0.32 | 2.98 | 0.0107 | 1.00 |
| C-pH | 3.06 | 1 | 0.32 | 9.68 | <0.0001 | 1.00 |
| $A^2$ | -1.11 | 1 | 0.31 | -3.61 | 0.0048 | 1.00 |
| $B^2$ | -0.89 | 1 | 0.31 | -2.89 | 0.0160 | 1.00 |
| $C^2$ | -1.48 | 1 | 0.31 | -4.80 | 0.0007 | 1.00 |
| AB | 0.082 | 1 | 0.41 | 0.20 | 0.8458 | 1.00 |
| AC | 0.28 | 1 | 0.41 | 0.68 | 0.5099 | 1.00 |
| BC | -0.033 | 1 | 0.41 | -0.079 | 0.9389 | 1.00 |

APPENDIX 38: Screening Experiment - ANOVA of protein solubility of mixed pea protein-carrageenan systems. *(Note: Design Expert V5.31 only displays probability values of significant factors (p < 0.05) )*

Dependent Variable: protein solubility

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | g/100 g | Numeric | 10.00 | 20.00 |
| B | Prot/Poly | g/100 g | Numeric | 20.00 | 100.00 |
| C | Prot/Nacl | g/100 g | Numeric | 10.00 | 30.00 |
| D | pH | g/100 g | Numeric | 4.00 | 9.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model | 748.04 | 5 | 149.61 | 302.57 | <0.0001 |
| Curvature | 1.29 | 1 | 1.29 | 2.60 | 0.1309 |
| Residual | 6.43 | 13 | 0.49 | | |
| Lack of Fit | 6.14 | 10 | 0.61 | 6.42 | 0.0764 |
| Pure Error | 0.29 | 3 | 0.096 | | |
| Cor Total | 755.75 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 0.70 | R-Squared | 0.9915 | |
| Dep Mean | 8.06 | Adj R-Squared | 0.9882 | |
| C.V. | 8.72 | Pred R-Squared | 0.9785 | |
| PRESS | 16.23 | Adeq Precision | 42.650 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 8.19 | 1 | 0.18 | | | |
| A-Prot Conc | 2.18 | 1 | 0.18 | 12.39 | <0.0001 | 1.00 |
| B-Prot/Poly | 0.60 | 1 | 0.18 | 3.43 | 0.0045 | 1.00 |
| D-pH | 6.02 | 1 | 0.18 | 34.24 | <0.0001 | 1.00 |
| AD | 2.25 | 1 | 0.18 | 12.80 | <0.0001 | 1.00 |
| BD | -0.60 | 1 | 0.18 | -3.41 | 0.0046 | 1.00 |
| Center Point | -0.63 | 1 | 0.39 | -1.61 | 0.1309 | 1.00 |

APPENDIX 39: Preliminary Optimization Experiment - ANOVA of protein solubility (g/100 g) of mixed pea protein-guar gum systems.

Dependent Variable: protein solubility

| Factor | Name | Units | Type | -1 Level | +1level |
|---|---|---|---|---|---|
| A | Prot Conc | g/100 g | Numeric | 15.00 | 25.00 |
| B | Prot/Poly | g/100 g | Numeric | 15.00 | 75.00 |
| C | pH | g/100 g | Numeric | 6.00 | 8.00 |

| Source | Sum of Squares | DF | Mean Square | F Value | Prob > F |
|---|---|---|---|---|---|
| Model (linear) | 306.56 | 3 | 102.19 | 14.73 | <0.0001 |
| Residual | 111.02 | 16 | 6.94 | | |
| Lack of Fit | 110.62 | 11 | 10.06 | 125.91 | <0.0001 |
| Pure Error | 0.40 | 5 | 0.080 | | |
| Cor Total | 417.58 | 19 | | | |

| | | | | |
|---|---|---|---|---|
| Root MSE | 2.63 | R-Squared | 0.7341 | |
| Dep Mean | 14.90 | Adj R-Squared | 0.6843 | |
| C.V. | 17.68 | Pred R-Squared | 0.5138 | |
| PRESS | 203.03 | Adeq Precision | 12.528 | Desire > 4 |

| Factor | Coefficient Estimate | DF | Standard Error | t for $H_0$ Coeff=0 | Prob > \|t\| | VIF |
|---|---|---|---|---|---|---|
| Intercept | 14.90 | 1 | 0.59 | | | |
| A-Prot Conc | 3.64 | 1 | 0.71 | 5.10 | 0.0001 | 1.00 |
| B-Prot/Poly | 0.82 | 1 | 0.71 | 1.15 | 0.2684 | 1.00 |
| C-pH | 2.93 | 1 | 0.71 | 4.11 | 0.0008 | 1.00 |