# SOY PROTEIN-XANTHAN GUM INTERACTION: STABILITY AND RHEOLOGY

by

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(ABSTRACT)

This study investigated the effects of ionic strength, pH, gum concentration, and protein type on protein - xanthan gum interactions. Commercial soy sauce and tamari sauce as well as model systems of soy protein isolate and whey protein concentrate were the sources of protein used for evaluation with xanthan gum.

Preliminary research indicated that when either soy sauce or tamari sauce were mixed with xanthan gum, stable solutions with notable viscosity synergisms resulted. The soy protein and whey protein systems were subsequently prepared with a range of 0 to 5% added sodium chloride. Results indicated that an equilibrium existed between proteins and xanthan gum such that increased sodium chloride initially increased solution stability; but when in excess, the sodium chloride led to a loss of protein - xanthan gum solution solubility and in some cases to precipitation. Precipitation was also noted at the pH extremes of 2,3, and 9 and when xanthan gum was present in excess, or at 0.25%.

The effects of sodium chloride, protein type, and pH on

the rheological parameters of model solutions were also examined. Higher sodium chloride levels yielded greater viscosity synergisms. Those solutions made with intact protein were generally higher in apparent viscosity than similar solutions made with hydrolyzed protein. Solutions at pH 5 were generally higher in viscosity than were similar solutions at pH 7.

Several factors that appeared to affect the stability, solubility, and the rheological parameters of protein - xanthan gum solutions were sodium chloride concentration, gum concentration, pH, and protein type.

ACKNOWLEDGEMENTS

As with any large endeaver, rarely creditable to one person alone, this work was accomplished through the contributions of several people. I would like to express my appreciation to all of my associates who have contributed in innumerable ways. A few people for whom I have special thanks are fondly mentionned below.

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The greatest thanks of all goes to my husband, Mohammad R. Ashayeri, who was a source of inspiration, encouragement, and joy and who contributed both tangibly and intangibly to the completion of this work.

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# I. INTRODUCTION

Soy protein is a major component of the diet in many countries. Though a relatively inexpensive protein source, soy has not found ready acceptance in the United States. For the most part soy protein is not considered by Americans to be palatable. In an effort to improve the acceptance of soy protein, scientists and food processors have attempted to incorporate soy into textured food products.

Many traditional foods such as pasta are textured products. In order to produce these commonly accepted products and newer ones of the future, food scientists must know the properties of each ingredient and understand how ingredients interact. It is the functional characteristics of ingredients which make it possible for them to perform successfully as a food product. Quality can be improved by utilizing functional properties of ingredients so that the aesthetic, nutritive, and/or economic values of the product as a whole are improved.

Texturized foods frequently involve the interaction of protein and polysaccharides. Though the reactions between proteins and polysaccharides have been studied for a number of years, little has been published specifically about xanthan gum. The work published on protein-xanthan gum interactions, generally found that precipitation resulted. Jackman (U. S. Patent, 1979) observed the interaction of xanthan gum, or a combination of xanthan gum and

carboxymethylcellulose (CMC), with orange juice protein in a 35% juice drink. She found that xanthan gum alone with the juice resulted in precipitation of the orange juice protein. The addition of a xanthan gum-CMC mixture to the juice inhibited precipitation. Heubner and Wall (1979) noted the interaction between xanthan gum and wheat gluten. They reported that the addition of xanthan gum to the protein resulted in precipitation of the protein.

Personal communication (Saltmarch, 1982) indicated a unique reaction between soy protein and xanthan gum in a high salt, liquid soy product, soy sauce. Rather than precipitation resulting, an unusual viscosity synergism occurred upon mixing soy sauce and xanthan gum. This led to the idea that soy protein and xanthan gum could possibly be used together in liquid, protein containing products. The relatively low cost and ample supply of each of these ingredients makes the potential for using them together interesting not only to food scientists but to manufacturers. This study examined the soy protein-xanthan gum systems to determine the effects of ionic strength, protein type, and pH. The specific objectives of this research were:

1) To evaluate the rheological parameters of soy sauce-xanthan gum systems and tamari sauce-xanthan gum systems.

2) To evaluate the effect of varying ionic strength on the stability and viscosity of intact and hydrolyzed soy protein-

xanthan gum solutions, using sodium chloride.

3) To evaluate the effects of three levels of xanthan gum on the stability and viscosity of soy protein solutions at five levels of sodium choride.

4) To use gel filtration to determine whether xanthan gum and soy protein actually complexed when blended together in a solution.

5) To evaluate whether protein type influenced the xanthan gum-protein interaction by evaluating intact whey protein for stability and % soluble protein in the presence of xanthan gum.

# II. REVIEW OF LITERATURE

## A. SOY PROTEIN ISOLATES

### 1. Composition

The typical soy protein isolate contains 90 to 97% protein, 4 to 7% moisture, 3 to 5% ash (minerals), 0.2 to 1% fat, and fiber. The protein content is calculated using a nitrogen conversion factor of 6.25. While there is some recent controversy over the appropriateness of 6.25, it has become the standard. As can be seen, the range of protein content is quite large. Information for any experiment using soy protein isolate should be specific for that particular isolate. Table 1 illustrates the composition of the Supro 710K soy protein isolate used throughout the research. Table 2 gives the amino acid composition of Supro 710K. The protein components of soy protein isolates are comprised primarily of 7S and 11S globulins. As seen in Table 3, the 11S fraction has a relatively high molecular weight of 350,000.

### 2. Structure

Both the 7S and 11S fractions form disulfide linked polymers which result in insolubility, turbidity and increased viscosity ( Wolf, 1970 ). The 7S and 11S globulins are characterized by complex quaternary structures which easily undergo association-dissociation reactions. Hermansson (1979) studied the denaturation of a soy protein isolate using a differential scanning calorimeter . Hermansson's work showed that salt appeared to stabilize the

soy protein isolate structure by limiting dissociation and denaturation. This finding was confirmed by Wolf and Cowan ( 1975 ) and Catsimpoolas ( 1970 ). The 11S proteins can be broken down to 7S and 2S or 3S forms. Research by Catsimpoolas ( 1970 ) indicated that the 11S protein consisted of 12 subunits per molecule if no disulfide crosslinks occurred. A dimeric structure of two identical monomers made up of six subunits each was proposed by Catsimpoolas for the 11S molecule. Two conditions causing the breakdown of the 11S quaternary structure were pH extremes and temperatures above 80 $^{o}$C.

Table 1 . Composition of Supro 710K Soy Protein Isolate

| Component | | Percent |
|---|---|---|
| protein | | 85.2 |
| fat | | 5.4 |
| moisture | | 5.3 |
| ash | | 3.7 |
| | sodium | 1.3 |
| | phosphorus | 0.8 |
| | calcium | 0.2 |
| | fiber | 0.1 |
| | potassium | 0.05 |

Torun, 1979

Table 2 . Percentage Amino Acid Content of Supro 710K Soy Protein Isolate and Whey Protein Concentrate

| AMINO ACID | SOY | WHEY |
|---|---|---|
| alanine | 3.46 | - |
| arginine | 6.39 | - |
| aspartic acid | 9.32 | - |
| cystine | 1.37 | 1.25 |
| glutamic acid | 16.03 | - |
| glycine | 3.46 | - |
| histidine | 2.21 | - |
| isoleucine | 3.97 | 3.05 |
| leucine | 6.63 | 6.53 |
| lysine | 5.40 | 5.47 |
| methionine | 1.14 | 1.12 |
| phenylalanine | 4.43 | 2.05 |
| proline | 4.49 | - |
| serine | 4.38 | - |
| threonine | 3.21 | 3.09 |
| tryptophan | 1.17 | 1.37 |
| tyrosine | 3.11 | 4.80 |
| valine | 3.98 | 3.25 |
| ammonia | 1.73 | - |

Torun, 1979

Table 3. Components and Molecular Weights of Two Major Soy Protein Fractions

| Fraction | Component | Molecular Weight |
|---|---|---|
| 7S | Beta- Amylases | 61,700 |
| | Lipoxygenases | 102,000 |
| | Hemagglutenins | 110,000 |
| | 7S Globulin | 180,000 - 210,000 |
| 11S | 11S Globulin | 350,000 |

Wolf , 1970

## 3. Functional Properties

### a. Solubility

#### 1) Mechanical Effects

Shen (1981) did an in-depth study of soy protein isolate solubility. He found that soy was unique in that the percentage of soy in solution was not effected by the concentration of soy. For other proteins, the percentage of soluble protein decreased as the solution became saturated. Shen observed that for a given soy protein isolate, the percentage of soy in solution remained constant whether the total soy concentration was 1 or 18%. Shen also observed that different soy protein isolates had different percentages of solubility. The soy protein isolates studied by Shen (1981) ranged from 30 to 80% solubility.

Shen (1981) observed that the method of blending effected the percentage of soy that would solubilize. He noted that maximum solubility resulted after blending for 100 minutes in a shaker at 25 $^{o}$C. Again, the maximum percent soluble protein depended on the particular soy protein isolate used and ranged from 30 to 80% solubility. According to Shen (1981), the relatively long shaking time helped ensure that the soy would be in a steady-state before using. Even given the long shaking time, there remained one soy protein isolate fraction that was insoluble. Which fraction remained insoluble was not stated.

2) Ionic Effects

Typically low levels of sodium chloride and other salts had the effect of salting-in a protein. Salting-in is a result of increased protein solubility. Higher salt levels usually result in decreased protein solubility, or salting-out. The effects of salt on soy protein isolate were just the opposite. Work by Shen ( 1981 ) illustrates this.

Shen (1981) investigated the effects of NaCl, NaI, $Na_2SO_4$ and on the solubility of native and hydrolyzed soy protein isolate. Results are shown in Figures 1 and 2. He found that the native, as well as the denatured, soy protein was first salted-out then salted-in. Salt concentrations of 0.1 molar caused the maximum protein precipitation. Salt levels greater than 0.1 molar and 0.2 molar resulted in a salting-in of native and denatured soy protein isolate, respectively. Theory suggests that exposed hydrophobic surfaces on the protein were responsible for the salting-out (aggregation) of both native and denatured soy protein isolates ( Shen,1981 ).

It is apparent (Shen, 1981) that salts effect soy proteins structurally; Shen proposed that increasing salt concentrations reduced the hydrophobic surface area of soy protein. By reducing the hydrophobic surface area of soy protein, protein - protein association was reduced; hence salting - out was reduced.

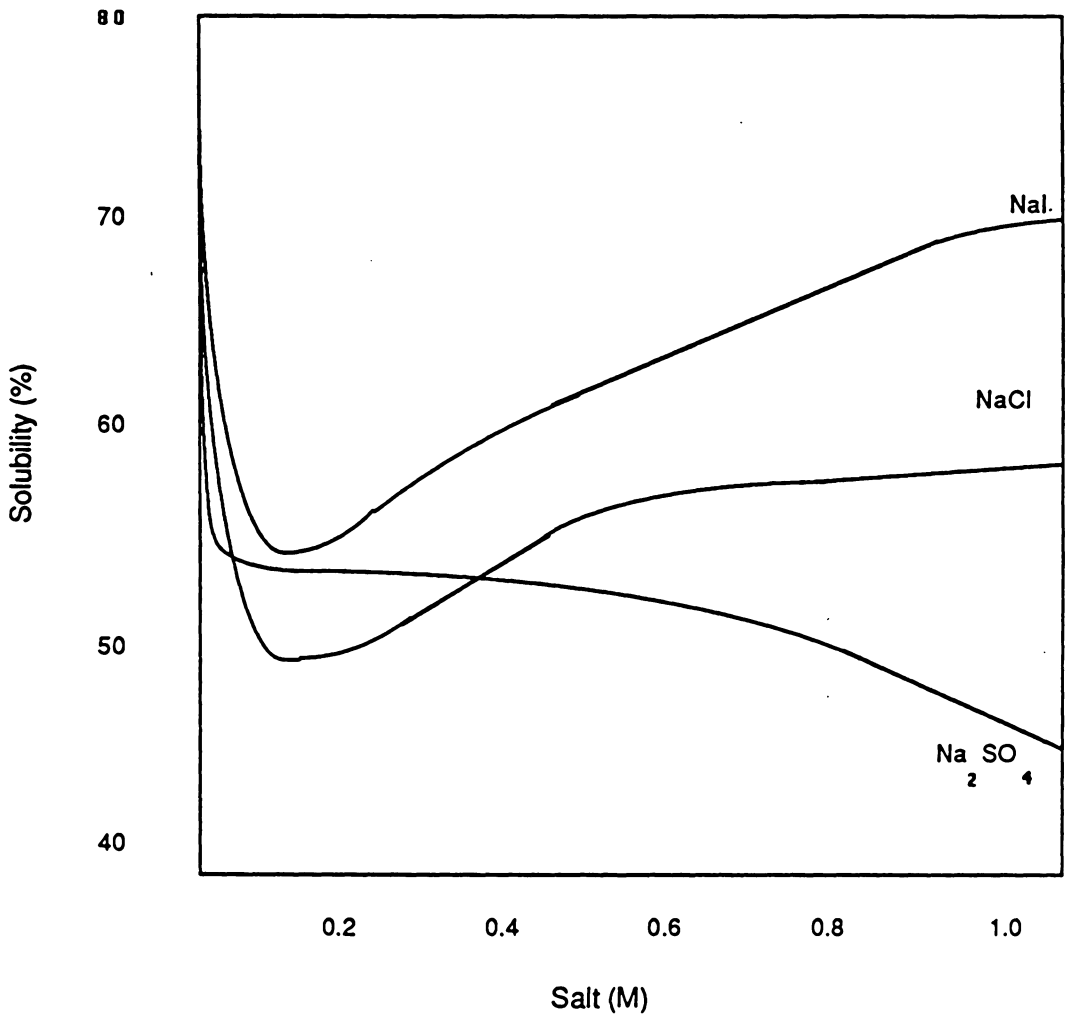



**Figure 1. The Effect of Various Salts on the Solubility of Intact Soy Protein Isolate (Shen, 1981)**

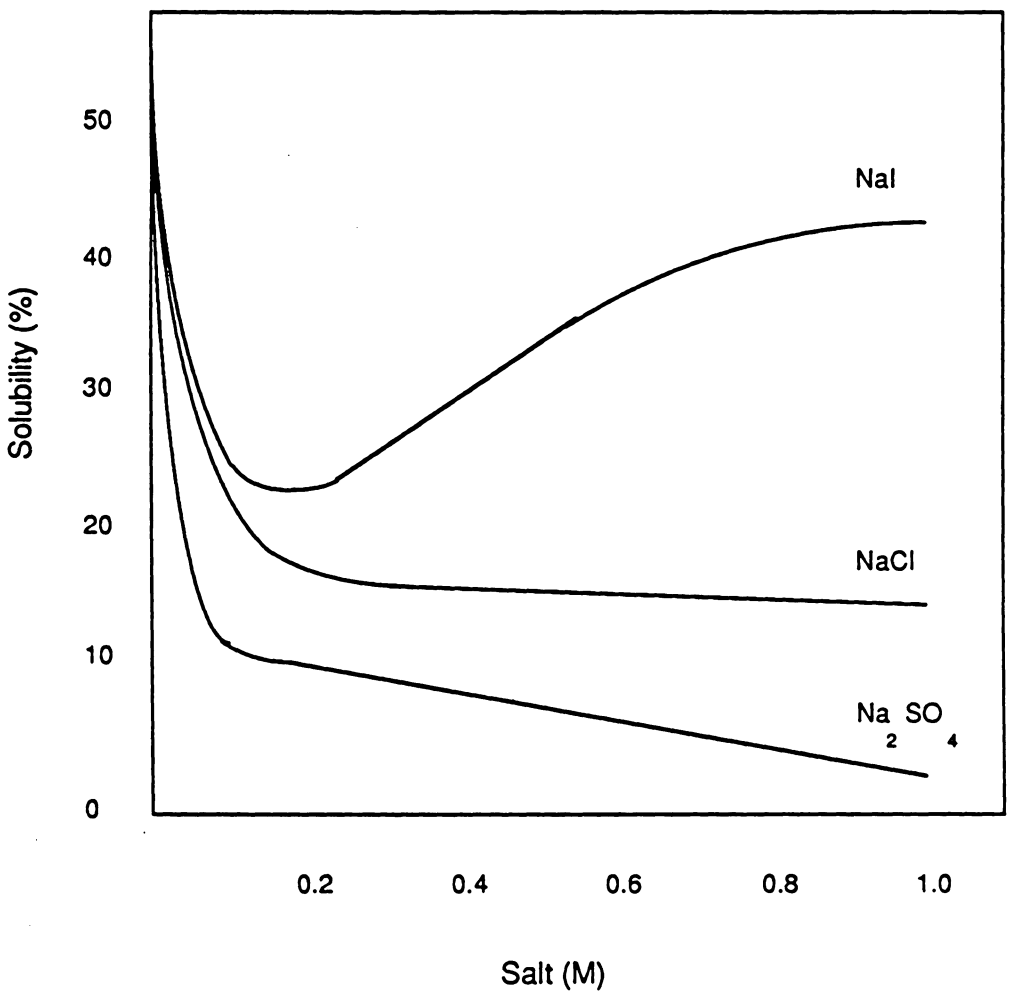



**Figure 2. The Effect of Various Salts on the Solubility of Hydrolyzed Soy Protein Isolate (Shen, 1981)**

In comparing the effects of salt on native and denatured soy protein isolate, Shen (1981) noted that in general, salt had twice the salting - out effect on the denatured soy as on the native soy. The fact that the denatured soy was salted - out more, indicated denatured soy had more exposed hydrophobic groups. In that the denatured soy is a smaller molecule than the native soy, it is to be expected that more of the hydrophobic areas would be exposed. With the larger native proteins, salt led to more intramolecular association, which in turn resulted in reduced hydrophobic surface area and less salting - out.

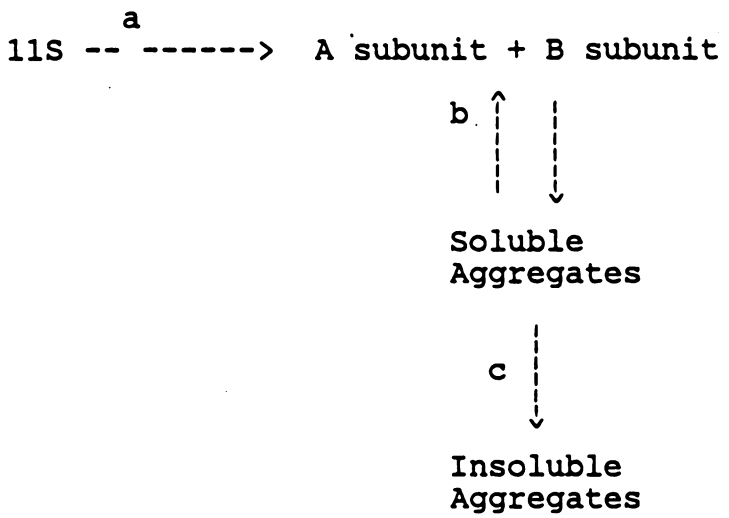
According to Shen (1981), sodium ions would not be expected to bind to soy proteins. Therefore, the net charge of the soy protein would not be altered by the presence of sodium ions. On the other hand, chlorine ions are known to have a salting - out effect on soy protein. The chlorine ions bind to soy protein, changing the protein's net charge. The effect is a reduction of the dipole moment. At the point where the net charge on the protein is zero, the protein is least soluble.

Hermansson (1979) also examined the effects of sodium chloride on soy protein isolate, using a differential scanning calorimeter technique. Hermansson, like Shen, found that the degree of soy protein aggregation increased as salt concentrations increased from 0.1 molar to 0.2 molar. He suggested the increased aggregation was due in part to the

dimerization of the 7S fraction. Between a 0.2 molar and a 2.0 molar concentration of sodium chloride, soy protein aggregation diminished; the 7S fraction is reduced to a monomer at the higher sodium chloride concentration. The stabilization of soy protein isolate's quaternary structure is a probable explanation of why the aggregation decreases at the higher salt concentrations (Koshiyama , 1968; Hermansson, 1979). The salt appeared to stabilize the protein against dissociation and denaturation.

Hermansson (1979) found the effect of the salt was most pronounced when the pH was outside the protein's isoelectric region, 4.5 to 5.0. At the extremes of pH, below pH 3.0 and above pH 11.0, no aggregation of a 0.5% soy protein, 0.2 molar sodium chloride solution was observed. Hermansson suggested that this was because of intermolecular repulsion forces at the high net charge; thus favoring protein-solvent rather than protein-protein interactions. At pH extremes, salt had the effect of suppressing both denaturation and aggregation of the protein.

There is evidence supporting the hypothesis that soy proteins form soluble aggregates that later convert into insoluble precipitates. Wolf and Tamura (1969) studied heat denaturation of the native 11S soy protein. They proposed the following mechanism to explain their findings of soluble aggregates prior to the formation of insoluble precipitates.




The reaction leading to the reversible formation of soluble aggregates has been suggested by several researchers, namely Catsimpoolas (1970), Hermansson (1979), and Wolf and Tamura (1969). Wolf and Tamura stated that they were unable to detect the B subunit, but suggested that was because reaction "b" occurred so rapidly. In reviewing Takagi et al. (1979), Shen reported that hydrophobic interactions were primarily responsible for the formation of aggregates that were soluble prior to intermolecular disulfide bonding. The disulfide bonding resulted in insoluble precipitates. On the other hand, Wolf (1970) suggested that disulfide linkages caused the aggregates to be soluble and their cleavage allowed exposed groups to undergo hydrophobic bonding leading to precipitation.

3) pH Effects

Soy protein becomes increasingly insoluble as the pH drops from 7.0 to 4.2. At pH 4.2 about 90% of the protein is

out of solution ( Seal and Lucas, 1980 ). Because the solubility decreases as the pH drops below 7.0, the viscosity increases.

Exposure to pH extremes results in disruption of the quaternary structure of the 7S and 11S proteins. The conversion is irreversible for the 11S fractions. It is usually reversible for the 7S fractions. However, Wolf (1970) found that the breakage of 7S proteins was irreversible when the 7S globulin was kept at pH 2.0 for a long time. Wolf did not define what was considered to be "a long time".

b. Viscosity

The viscosity of solutions of soy protein isolate increased exponentially with the concentration of protein. Viscosity can be used as an indirect method for determining solubility of protein.

Circle (1964) studied the ability of soy protein isolate to form gels. His results were vague, but a few generalities can be made from them. These are that the characteristics of protein gels were affected by intra- and inter- molecular bonding and crosslinking. Where the degree of crosslinking was not optimal, an undesireable gel structure resulted. Circle suggested gel structure was also affected by the presence of salt. Salt created an environment suitable either to ionic bonding between charged amino acid sidechains on the

protein or to the development of salt bridges. Later, Seal and Lucas (1980) observed that soy protein isolates gelled at concentrations of 8% or greater. Findings about solutions of 8% soy protein do not necessarily contribute to understanding of the current research in which only 2% protein solutions were studied. At only 2% soy protein, solutions would not be expected to gel.

## B. XANTHAN GUM

### 1. Structure

Xanthan gum is a heteropolysaccharide with a molecular weight on the order of two million. It is believed that certain xanthan gum molecules can have a molecular weight of fifteen to thirty million. Each unit consists of two glucose moieties, two mannose moieties, and one glucuronic acid moiety. There is a 1-4 linkage of the two beta-D glucose units. Thus the main chain of xanthan gum is structured like cellulose. The terminal beta-D mannose is linked glycosidically to the 4 position of beta-D glucuronic acid which in turn is linked glycosidically to the 2 position of the alpha-D mannose moiety. This three sugar side chain of mannose-glucuronic acid-mannose is linked to the C-3 position of every other glucose unit in the main glucose chain. Half of the terminal D-mannose residues carry a pyruvic acid residue that is ketalically linked to the 4- and 6- positions. An acetyl group is linked to the non-terminal mannose moiety at the 6-position. The xanthan gum structure is illustrated in Figure 3.

It is theorized by some that xanthan gum orders itself in a helical structure. According to Kelco (1977), xanthan gum's helices align themselves. These areas of alignment have been termed " super junction zones ". The proposed structure of the " super junction zone " is shown in Figure 4. The result of this suggested structure would be to

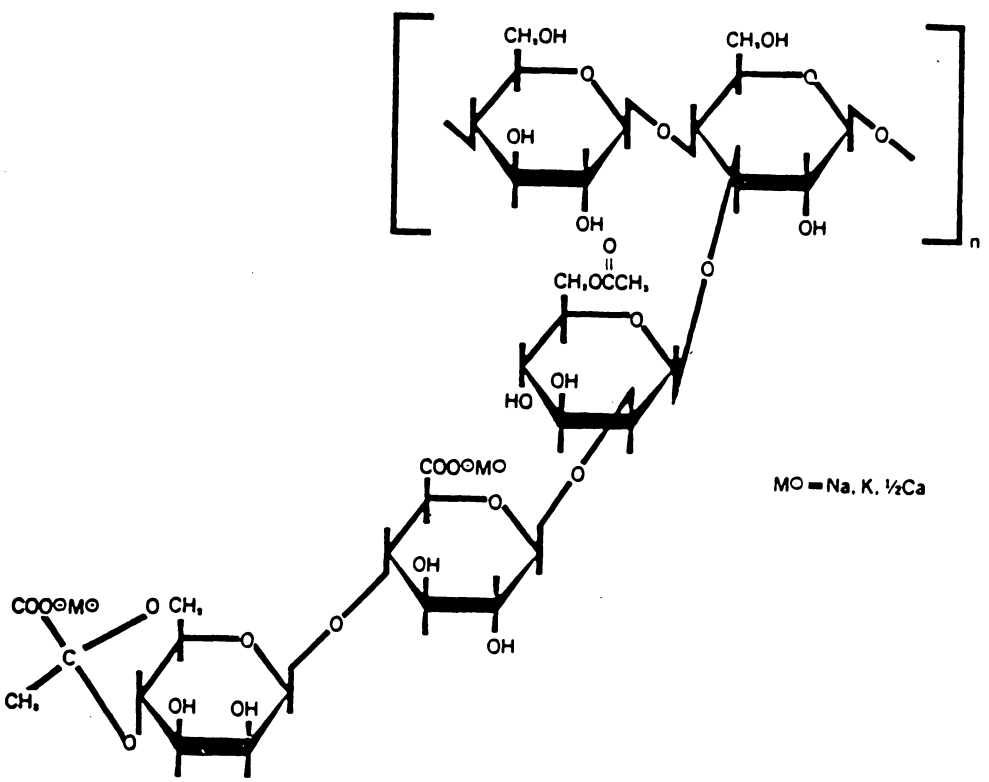



Figure 3. Xanthan Gum Structure

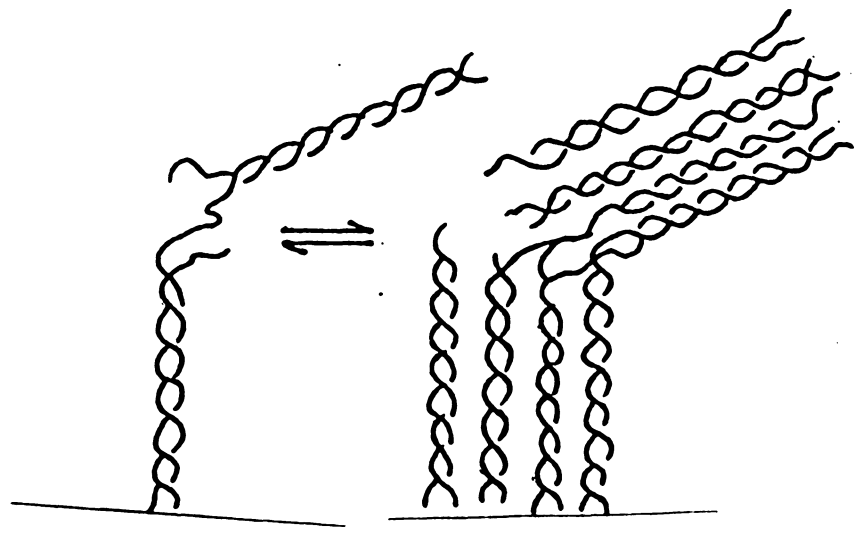


Figure 4. Helical Structure of Xanthan Gum: Super Junction Zone

Kelco, 1977

produce an extremely durable hydrophilic polysaccharide with unusual rheological properties.

2. Properties

One property of xanthan gum is that it is highly viscous at even low concentrations. Data from Kelco (1977) indicated a solution of 0.25% xanthan gum would have an apparent viscosity of 800, 300, 60, and 10 centipoise (cps) at 1, 10, 100,and 1000 reciprical seconds, respectively. The apparent viscosities expected for solutions of 0.25, 0.50, 1.0, and 2.5% xanthan gum are shown in Figure 5.

The gum is highly pseudoplastic, meaning that the apparent viscosity decreases as the shear rate is increased. Pseudoplastic solutions return to nearly their original viscosity when the shear stress is removed. Figure 5 illustrates rheograms for various xanthan gum solutions. In all such rheograms, the slope of the line is an index of pseudoplasticity. It can be seen that pseudoplasticity changed little as the xanthan gum concentration was changed from 0.25 to 2.5%. The fact that xanthan gum is pseudoplastic is explained by the concept of " super junction zones ".

A unique characteristic of xanthan gum is that its apparent viscosity is not extremely sensitive to increased temperatures. Morris (1979), using nuclear magnetic resonance spectroscopy of xanthan gum in water, demonstrated that the structure of xanthan gum was stable at temperatures

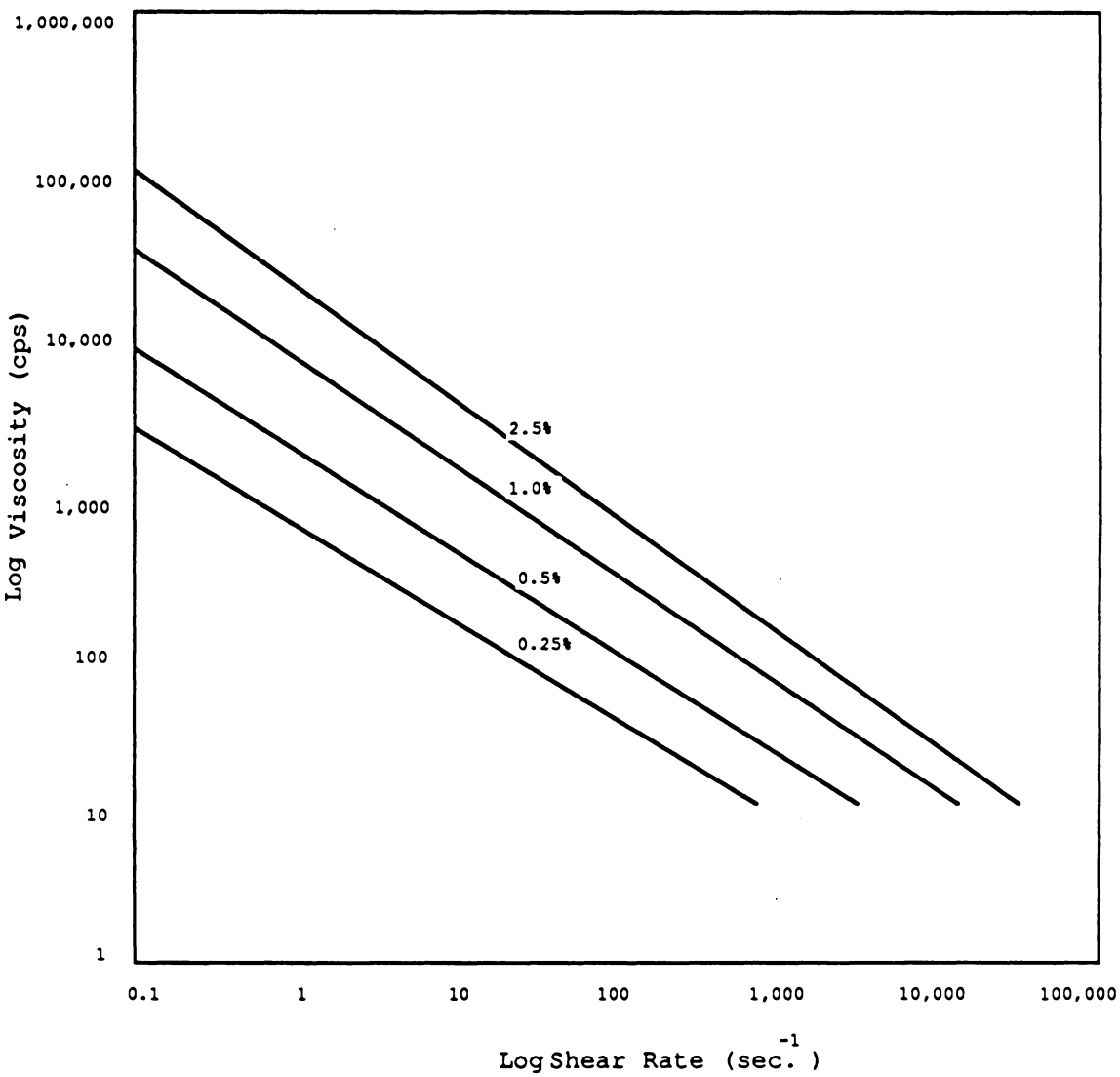



**Figure 5. The Apparent Viscosity of Xanthan Gum Solutions**

in excess of 100 °C. Kelco (1977) also published data supporting the fact that xanthan gum exhibited almost no change in viscosity as the exposure temperature increased from 1 °C to 60 °C.

A third xanthan gum property is its unusual stability to pH change. Figure 6 illustrates that xanthan gum's apparent viscosity remained relatively steady over a pH range of 1 to 11 ( Kelco, 1977 ).

Another of xanthan gum's properties is its stability in the presence of salt. At very low concentrations of xanthan gum, the addition of trace amounts of salt may cause some decrease in viscosity ( Rocks, 1971; Kelco, 1977 ). However, in moderate to high concentrations, xanthan gum is extremely stable to many salts ( Kovacs and Kang, 1977 ). It dissolves in as much as 15 % sodium chloride without significantly affecting the apparent viscosity. Data from Kelco indicated that the apparent viscosity of a 1 % xanthan gum solution was relatively unaffected by the addition of as much as 10 % sodium chloride. It was noted the initial apparent viscosity of a 1 % xanthan gum solution, without sodium chloride, was 860 cps and increased to 990 cps upon ninety days of storage. They reported the apparent viscosity of a similar solution with 5 % added sodium chloride was 1110 cps initially and dropped to 1090 cps after ninety days of storage. The addition of 10 % sodium chloride resulted in an apparent viscosity of 1120 cps initially; it decreased to 980 cps

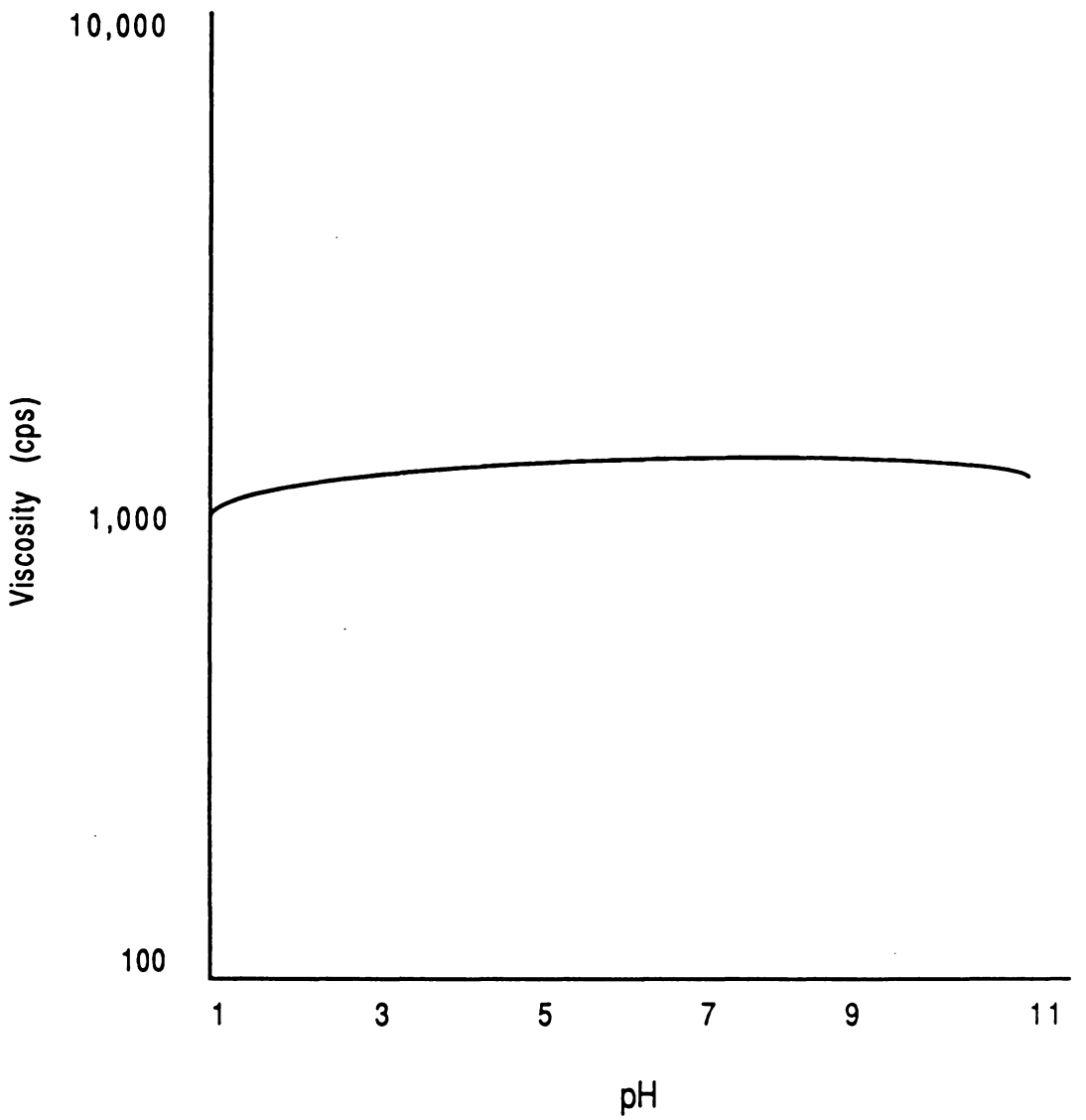



**Figure 6. Effect of pH on Xanthan Gum Viscosity**

after ninety days of storage. The data illustrated in Figure 7 indicates that the effect of sodium chloride on the apparent viscosity of xanthan gum becomes negligible as the salt content increases beyond 0.01 to 0.02 % (Kovacs and Kang, 1977). In most practical applications, the salt content would be sufficiently high to make the viscosity of xanthan gum essentialy independent of ionic strength.

While data from Kelco (1977) suggested that the viscosity of xanthan gum was stable to changes in salt concentrations, results of Smith et al. (1968) , as reported by Symes (1980), indicated that viscosity was contingent on the concentration of salt in conjunction with the degree to which the xanthan gum was pyruvylated. The results from Smith et al. (1968 ) do not contradict those of Kelco; they only add another factor. The more pyruvic acid side chains present, the more an increased salt concentration effected apparent viscosity. In the absence of salt, the opposite resulted; more highly pyruvylated xanthan gum exhibited the lower viscosity. Smith et al. (1968) proposed that at low ionic strengths, ionic repulsion between carboxylate groups acted to keep those groups separated. Sufficient salt then served to alter the ionic environment such that interchain repulsions were minimized and the xanthan gum would aggregate. As aggregation would occur, apparent viscosity would increase. Altering the degree of pyruvylation of

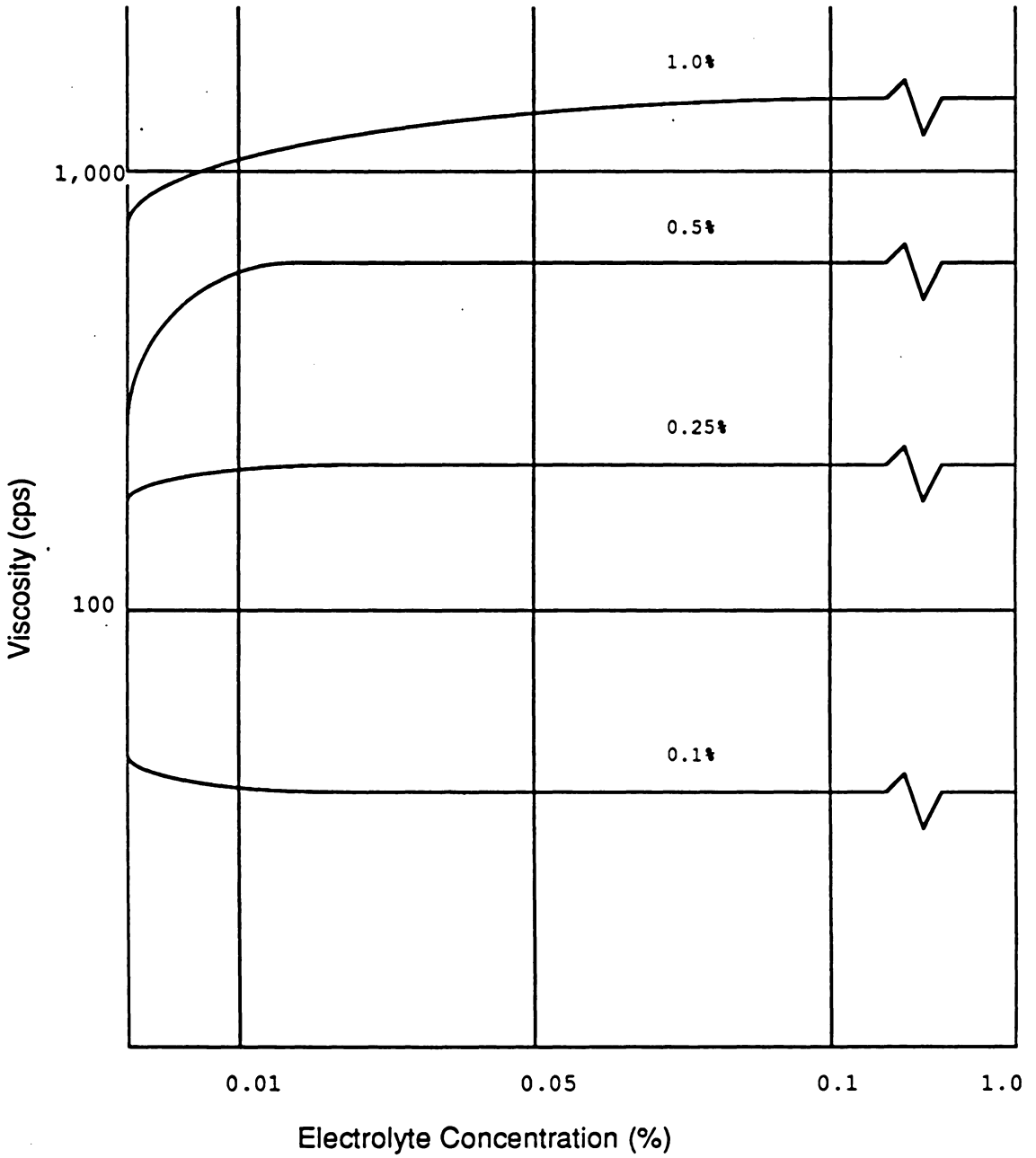



**Figure 7. Effect of Electrolyte Concentration on The Viscosity of Xanthan Gum ( Graham, 1977)**

xanthan gum was not the only way found to alter the charge on the gum. Protonation of the carboxylic acid groups could also minimize interchain repulsion. It was also suggested that protonation resulted in hydrogen bonding between xanthan gum chains. As salt increased it may have disrupted the hydrogen bonding thus creating smaller molecules and hence decreasing apparent viscosities.

The final property of xanthan gum is its resistance to enzymatic activity. According to Kelco (1977), the resistance to enzymatic activity is due to the nature of the sugar linkages as well as to the side chain substituents on the glucose backbone. The fact that xanthan gum is structured with a glucose backbone lends unusual strenth to the molecule.

## C. WHEY PROTEIN CONCENTRATE

### 1. Composition

Whey protein is a product remaining after casein and fat have been removed from milk. The major components of whey are lactose, salts, and water soluble proteins. The protein components include the following: beta-lactoglobulin, 50%; alpha- lactalbumin, 12%; immunoglobulins, 10%; bovine serum albumin, 5%; and proteose peptones, 23% (Evans and Gordan, 1980). Table 2 gives the amino acid content of the whey protein concentrate.

There are a number of reactive sites in whey protein molecules. The - lactoglobulin component contains sulfhydryl groups. The β - lactalbumin component contains four reactive tyrosine residues. It was reported by Kronman and Holmes (1965) that in the pH range of 6 to 2 at 25 °C only two of the four reactive tyrosine residues are "exposed" to dissolved sucrose or glycerol. Unfolding of the β - lactalbumin molecules resulted in all four tyrosine residues being exposed and reactive. Exposure to pH extremes results in the unfolding of whey proteins.

### 2.Solubility

The solubility of whey proteins is determined by pH and ionic strength of the environment. Whey is least soluble in its isoelectric range, which is 4.5 to 5.0. Sodium chloride concentrations between 0.1 and 0.3 molar function to improve

whey protein solubility somewhat. Higher sodium chloride concentrations have the effect of salting - out the protein. Whey has a compact globular structure. Upon denaturation, the protein unfolds, allowing either gel formation or precipitation.

3. Gelation

At concentrations of 7.5% or more, whey protein concentrate forms a gel when exposed to 100° C for ten minutes. Brief exposure to pH extremes improves the ability of whey protein concentrate to gel. The pH extremes cause the whey protein to unfold; this unfolding in turn exposes sulfhydryl groups, which contribute to protein aggregation. The addition of 0.1 to 0.3 molar sodium chloride results in maximum gel strength.

## D. RHEOLOGY

### 1. Introduction

Following is a review of rheology in general, the individual components of the model systems studied, and pertinent protein-polysaccharide interactions reported in the literature.

### 2. Rheology

#### a.General Principles

Viscosity is by definition the resistance of a substance to flow or shear. The unit of viscosity, the poise is the tangential force required to maintain a velocity of 1 cm/sec between two planes with areas of 1 cm and positioned 1 cm apart. As this force is often small, viscosity is commonly referred to in centipoise, or one hundreth of a poise. Two other terms need to be defined. One is shear stress which is force/area sheared. Shear stress is expressed in dynes/$cm^2$. The other term is shear rate, which by definition is velocity/ film thickness. Shear rate is expressed in reciprocal seconds ($sec^{-1}$). Therefore, viscosity is shear stress/shear rate. In terms of units, viscosity is defined as equation (1):

$$(dynes)(sec)/cm^2 = centipoise \qquad (1)$$

When shear stress ($\lambda$) is proportional to shear rate ($\gamma$), a

solution is said to be Newtonian. A formula which applies to Newtonian fluids is:

$$\lambda = \eta \gamma \qquad (2)$$

where $\eta$ = the flow behavior index or the index of pseudoplasticity of a solution. Only water and dilute solutions with viscosities very similar to water are Newtonian. Most hydrophilic solutions do not resemble water in their flow behavior and therefore are not Newtonian. While there are several types of Non-Newtonian fluids, the only type pertinent to the current research is the pseudoplastic one. A pseudoplastic fluid is one which decreases in apparent viscosity as the shear rate increases. When shear rate is plotted against the shear stresses, the resulting line is linear for Newtonian fluids, but is nonlinear for pseudoplastic fluids. This relationship is illustrated in Figure 8. Pseudoplastic fluids follow the Power Law Relationship which is described by the equation:

$$\lambda = m\gamma^{\eta} \qquad (3)$$

where $\lambda$ = shear stress
$m$ = consistency coefficient
$\gamma$ = shear rate
$\eta$ = flow behavior index

It can be shown through derivation of the Powers Law Model that:

$$n = \text{slope} + 1 \qquad (4)$$

Figure 9 illustrates the rheogram that would result from a

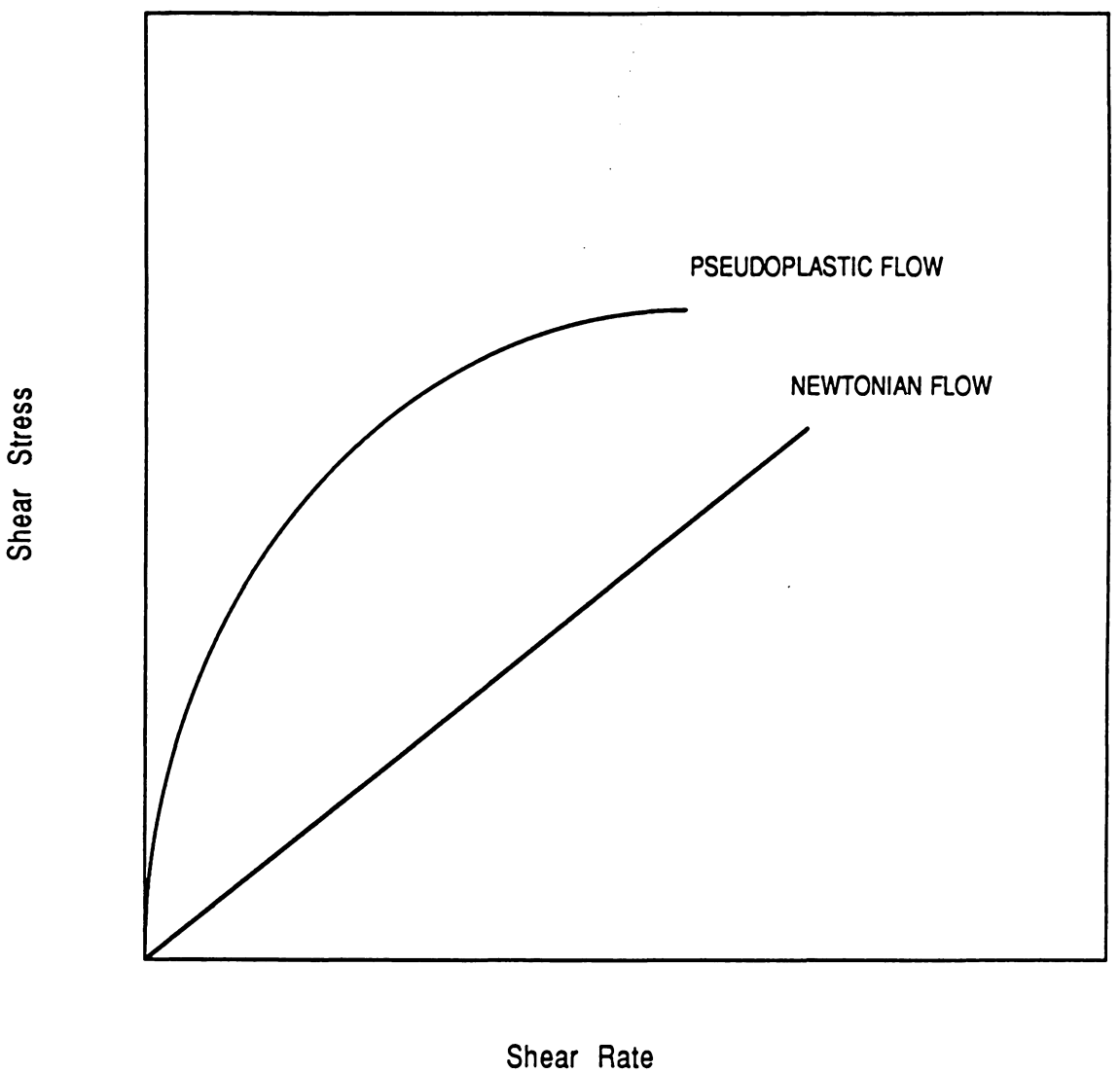



**Figure 8. Rheograms of Flow Behavior of Food Sols**

plot of the above equation. The log of the apparent viscosity is plotted against the log of the shear rate in reciprocal seconds. The slope is equal to n-1. That is to say that the flow behavior index, n, is equal to the slope + 1. For Newtonian fluids, n = 1 because the slope = 0. The slopes which result from plotting data points of Non-Newtonian fluids on a rheogram such as the one illustrated in Figure 8 are generally negative. Therefore, the smaller the value of n the more pseudoplastic the fluid. The consistency coefficient, m, is the viscosity at one $sec^{-1}$ as determined via linear regression analysis.

b. Rheology of Proteins and Polysaccharides

The flow properties of proteins and polysaccharides are governed by several factors. While molecular size is one such factor, molecular conformation is far more influential. The larger the dynamic volume, the greater the apparent viscosity. Highly branched molecules tend therefore to have a lower apparent viscosity than linear molecules of the same molecular weight. Charge also effects the molecular shape. Polyelectrolytes will be more expanded due to electrostatic repulsion than will non-ionic molecules. Everything else being equal, the more highly expanded molecules will have a greater apparent viscosity because they will have a larger dynamic volume. Molecular size, shape, and charge of proteins and polysaccharides can in turn be effected by such things as temperature, concentration, pH, ionic strength, and

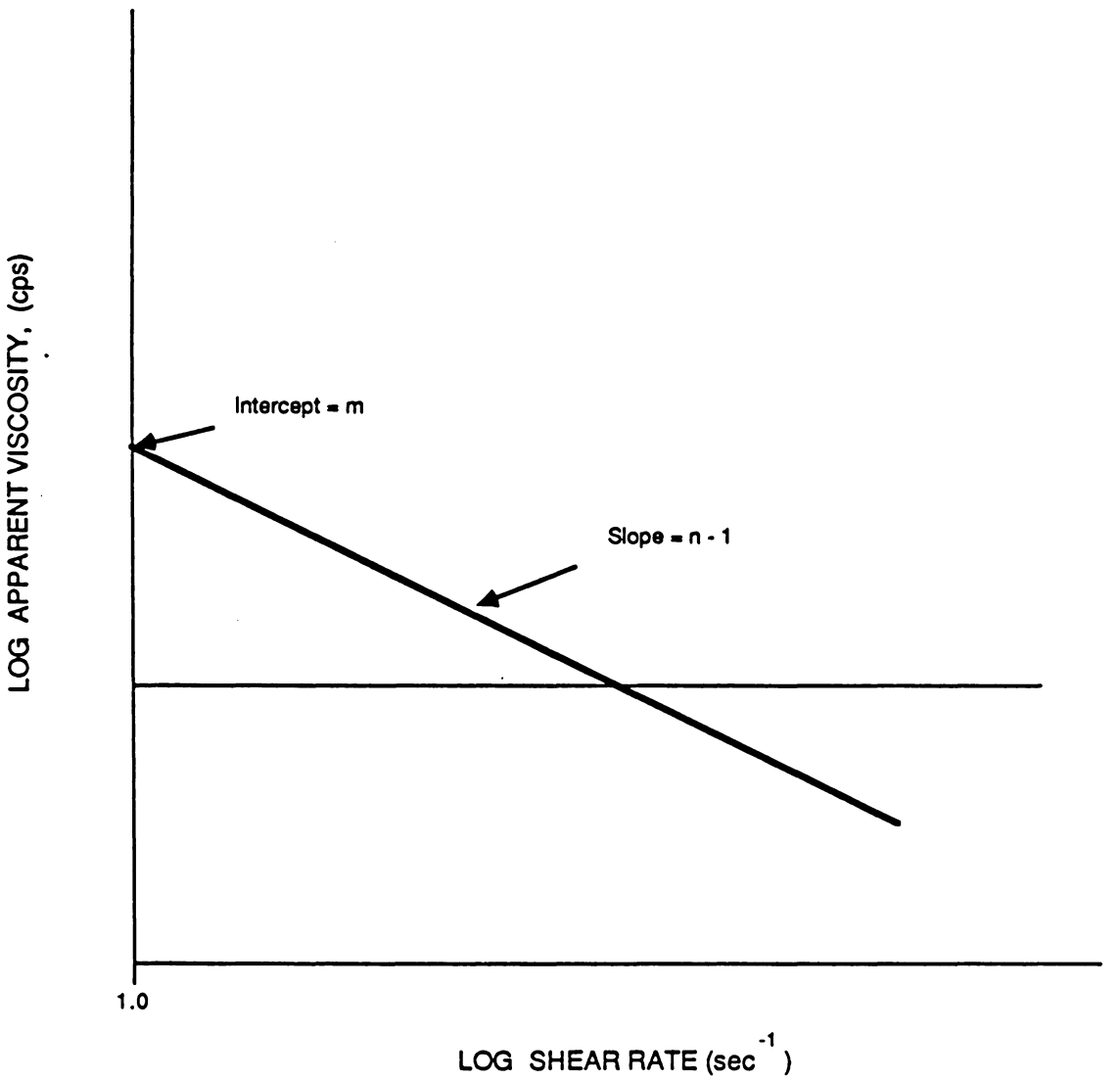



**Figure 9. Rheogram for Power Law Model**

the history of the protein ( Tung,1978).

c. Rheology of Xanthan Gum

At very low shear rates the apparent viscosity of xanthan gum is high. This is explained by the fact that the gum is of a large molecular weight, has a relatively long rodlike backbone of glucose units, and is charged. However, as the shear rate is increased, the apparent viscosity of xanthan gum decreases dramatically. With increased shear rate, the molecules of xanthan gum orient themselves, thereby offering less resistance to flow. When the shear is removed, the apparent viscosity increases to nearly that of the original gum solution. Figure 4 illustrates the concept of super junction zones which are believed to be responsible for increased viscosity as shear is decreased ( Kelco, 1975)

Measurements of apparent viscosity are dependent on the equipment used and the environmental conditions at the time of measurement. The Brookfield RVT Rotoviscometer with UL Adaptor imposes shear rates from 0.61 to 122.36 $sec^{-1}$. In this range of shear, a 0.25% xanthan gum solution is expected to range from approximately 800 to 10 centipoise (cps) , respectively (Kelco,1975). Figure 10, replicated from Kelco, illustrates this. According to Kelco (1975), the expected consistency coefficient, (m), of a 0.25% xanthan gum solution was approximately 800 cps.

Because of the gum's ability to support microbial

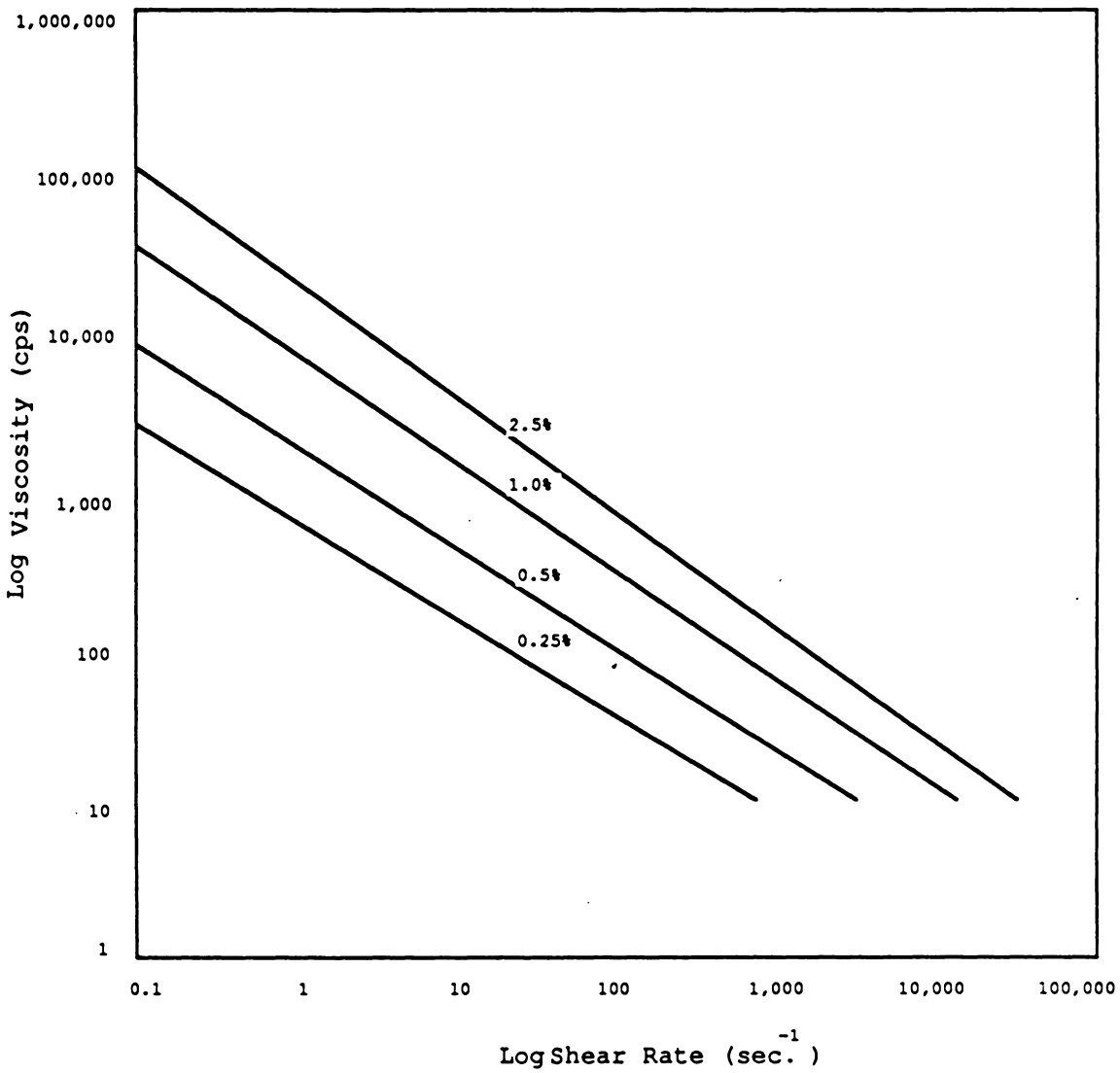



**Figure 10. The Apparent Viscosity of Xanthan Gum Solutions**

growth, Kelco preserved xanthan gum solutions with the addition of 0.1% formaldehyde. This addition had minimal effect on the gum's viscosity. Xanthan gum is compatible with most preservatives. An exception to this is quaternary ammonium compounds, which should not be used to preserve the gum.

## E. PROTEIN - POLYSACCHARIDE INTERACTION

### 1. Introduction

Most of the work on interactions between hydrophilic polysaccharides and proteins has been with acidic polysaccharides, including carboxymethylcellulose ( CMC ), xanthan gum (XG), and others. This review will first discuss published studies involving xanthan gum. The review will then discuss work done on protein - polysaccharide interactions where the hydrophilic polysaccharide was one other than xanthan gum.

### 2. Protein - Xanthan Gum Interactions

No studies have been published on the interaction of xanthan gum with soy protein. One of the few studies that has included xanthan gum was that of Jackman (1979). Jackman mixed xanthan gum in orange drink containing 35 % orange juice. The solution was acidic; however, the pH was not stated in the publication. The orange drink was only 0.19 % protein. Even with this low concentration of protein, xanthan gum - protein complexes flocculated in the drink. Jackman did not analyze the composition of the flocculent; rather, she assumed it to be both protein and xanthan gum. It would have been valuable if Jackman had identified and quantified the flocculent, but she did not.

Jackman (1979) found that by adding a XG-CMC combination to the orange juice drink, precipitation and flocculence were

prevented. She attributed this lack of precipitate to the probable interaction of CMC with the orange proteins. She proposed that CMC and the orange juice proteins preferentially formed a soluble complex. Once complexed with the CMC, the protein was not available for association with the XG.

Heubner and Wall (1979) studied protein polysaccharide interactions with xanthan gum in an attempt to find a polysaccharide that would increase the stability of soft red winter wheat. They brought solutions of red winter wheat to levels of 0.2 and 0.6 % xanthan gum. They also added xanthan gum to a dough made from the same red winter wheat. It was found that while xanthan gum caused increased stability of the wheat - gum dough, as evidenced on a farinograph, it caused the wheat in the solution to precipitate. The precipitates were stringy. Because of extreme precipitation, neither turbidity nor viscosity measurements were possible. Xanthan gum reacted with protein solutions containing as little as 0.002 % protein. Heubner and Wall (1979) proposed that hydrogen bonding was responsible for the interaction between XG and the wheat protein. They noted the carboxylate groups on XG were mainly associated and therefore would not react significantly with the $NH_4$ groups on the protein. They suggested, however, that through hydrogen bonding, the carboxyl groups could combine with amide or other side groups of the wheat protein.

When soy sauce was combined with xanthan gum, no precipitation resulted ( Saltmarch, personal communication ). Soy sauce contains approximately 12 to 14% soy protein. The results of work by Jackman (1979) and Heubner and Wall (1979) might lead one to expect a precipitate in the soy sauce - xanthan gum solutions; the results of both these researchers having indicated that xanthan gum caused proteins to precipitate. Soy sauce is high in ionic strength. It is this high ionic strength that might have affected the interaction between soy protein and xanthan gum. In an effort to test this hypothesis regarding the effects of xanthan gum on soy protein in an ionic environment, the current research was undertaken.

Morris et al. (1979) and Dea (1979) studied the interaction between xanthan gum and galactomannans. While galactomannans are not proteins, the interactions proposed by both research groups is pertinent to the current research. Both researchers proposed that the smooth, unbranched segments of galactomannans complexed with xanthan gum such that the galactomannan was incorporated into the helical structure of the xanthan gum. The less branched the galactomannans were, the more readily they complexed with xanthan gum. It is interesting to note that a viscosity synergism was noted when the galactomannans reacted with the xanthan gum.

### 3. Polysaccharides Other Than Xanthan Gum and Their Interactions With Protein

#### a. Soy Protein Interactions

Ganz (1974) observed the interaction of soy protein with CMC. Upon mixing 0.5 % CMC in a 0.5 % soy solution, Ganz observed a phenomonal viscosity increase. He found that reducing the pH of the stable CMC - soy solutions resulted in precipitation. Ganz hypothesized that the carboxyl groups of the CMC combined with the positively charged protein residues to form soluble complexes. Further, he reported that where stable soy - CMC complexes were formed, even heat would not cause precipitation. Ganz thereby supported the theory posed by Hidalgo and Hansen (1969). This theory was that the protein and polysaccharide react to form a complex that is soluble.

#### b. Other Proteins and Their Interactions With Hydrophilic Polysaccharides

Hidalgo and Hansen (1969) observed precipitation of approximately 100 % of the beta -lactoglobulin initially in solution with 0.2 % CMC at low ionic strenth. It was observed that pH effected the degree of precipitatin. Maximum precipitation occurred at pH 4.0, whereas no precipitation occurred at pH's below 2.5 or above 7.0. They examined the constituents of the insoluble complex, which precipitated, at pH 4.0 and ionic strength of 0.05. The maximum amount of precipitate was a function of the amount of

CMC added to the beta - lactoglobulin. At the point of maximum precipitation, they observed that 93 % of the CMC was bound to the protein in the precipitate. Increasing the ionic strength of beta -lactoglobulin - CMC solutions resulted in decreased amount of precipitation. Upon increasing the ionic strength to 0.25, electrophoretic patterns showed the beta - lactoglobulin and gum to be completely independent of one another. A beta - lactoglobulin - CMC precipitate, formed at relatively low ionic strenths, would actually be resolubilized by increasing the ionic strength to 0.25.

Hidalgo and Hansen (1969) also examined the effects of different types of CMC on beta -lactoglobulin. Increasing levels of carboxylation, as seen in 4-HP, 7-HP, and 9-HP CMC, were studied. The addition of 7-HP and 9- HP CMC, beyond the concentration that resulted in maximum precipitation, resulted in less and less precipitation of the protein. Ultracentrifugation patterns of 7-HP CMC - protein solutions indicated the presence of fast moving molecular complexes. This was evidence that the protein and 7-HP CMC did not disassociate, but rather formed soluble complexes. Hidalgo and Hansen ( 1969 ) suggested that peptization of the complex was what resulted in its being soluble.

The theory posed by Hidalgo and Hansen (1969) was similar to that posed later by Ganz; this was that a positively charged protein would interact with negatively charged regions of an anionic gum and form insoluble complexes.

Being insoluble, these complexes would precipitate. Hidalgo and Hansen (1969) proposed also that adding an anionic gum in excessive quantities could result in a soluble complex being formed between the gum and the protein. This could be explained by the fact that adding an excess of negatively charged molecules would actually alter the ionic environment. Hidalgo and Hansen suggest that in an environment with excessive polysaccharide, an insoluble complex would form first. Then the protein molecules would redistribute in such a way as to uncoil somewhat. This would lead to increased hydration, thus increased solubilization.

Imeson et al. (1977) observed the effects of several polysaccharides on the stability of myoglobin. To do this they used a differential scanning calorimeter. When myoglobin alone was adjusted to a pH of 6.0, at a low ionic strength, it precipitated. When myoglobin was mixed with alginate, pectate, or CMC, and brought again to pH 6.0, at a low ionic strength, no precipitation resulted. Even heat would not cause the myoglobin to precipitate out of solution with the polysaccharide. Imeson et al. (1977) analyzed solutions of 5 % myoglobin and 5 % myoglobin - 1 % pectate. Results indicated that the polysaccharide decreased the denaturation temperature byabout 5 $^{o}$C. Alginate, CMC, and dextrin also served to lower the denaturation temperature of myoglobin. The observed decrease in denaturation temperature

was less pronounced as the ionic strength was increased. The authors suggest that the smaller drop in the denaturation temperature at higher ionic strengths indicated diminished interaction between the protein and the polysaccharide. According to Ledward (1979), following denaturation, the available number of positively charged protein residues may increase. This is because the basic groups are liberated. The flexibility of the random coil in the denatured state permitted configurational adjustments to maximize the interaction; thus, more stable complexes were formed. In summary, it can be said that polysaccharides accelerate protein denaturation which in turn allow the formation of relatively stable complexes.

Imeson et al. (1977) used a Perkin - Elmer 124 double beam recording spectrophotometer to determine the spectra of some myoglobin and myoglobin - polysaccharide solutions. Results indicated that the presence of anionic polysaccharides decreased the absorption of the myoglobin spectrum in the Soret ( approximately 400 nanometers ) region. The ability of alginate to distort the myoglobin structure increased with decreased pH.

Heubner and Wall (1979) observed the effects of many polysaccharides on soft and hard red winter wheat. One gum, aside from xanthan gum, that they studied was an acidic polysaccharide originated from Arthrobacter viscosus. They found that, when wheat proteins were combined with this

polysaccharide, flocculence occurred. This same polysaccharide, when deacylated, did not cause flocculence. Heubner and Wall (1979) explained this phenomenon by suggesting that the acetyl groups of the polysaccharide make it more hydrophobic. Hydrophobic groups probably reacted with the non-polar residues of the protein. Removal of the acetyl groups exposed hydroxy groups and rendered the polysaccharide more hydrophilic, with more groups to react by hydrogen bonding to the protein. There seems to be a contradiction in the Heubner and Wall proposal. Heubner and Wall suggest hydrogen bonding as the possible cause for precipitation. However, they then suggested it was hydrogen bonding between the deacetylated polysaccharide and the protein which prevented precipitation and resulted in increased viscosity. Perhaps what makes the distinction between increased viscosity and precipitation is the extent of the bonding. Some hydrogen bonding of the gum and protein probably result in an increased apparent viscosity. More extensive bonding may result in large particles which precipitate due to high molecular weight.

Cluskey et al. (1971) studied casein with CMC and concluded that no precipitation resulted. Both casein and CMC are negatively charged. No precipitate formed until calcium was added; the charge on the calcium allowed for ionic crosslinking.

Ganz (1974) studied the effects of adding CMC to two gelatins with different isoelectric ranges. The results of mixing the gelatins and the CMC were increased viscosities. Neither of the proteins precipitated in the isoelectric range. Decreasing the pH below the isoelectric range first caused a viscosity decrease; a further drop in pH resulted in turbidity and then precipitation. Ganz noted that sodium chloride tended to stabilize the solutions up to a certain unspecified drop in pH.

A study which laid much of the groundwork for gelatin - polysaccharide interactions was that of Woodside et al. (1968). Complex formation was determined by turbidity, which in turn was determined spectrophotometrically. Unlike other studies, this one reported that even some neutral polysaccharides resulted in precipitation when combined with gelatin. One of the neutral polysaccharides which formed an insoluble complex with gelatin was glycogen. The formation of gelatin - glycogen complexes increased when phosphorylated glycogen was used. Upon reacting the phosphorylated glycogen with gelatin, turbidity increased to a maximum then decreased. The degree of turbidity in the phosphorylated glycogen - gelatin solution was found to be dependent on protein concentration. The authors did note that increased precipitation a high gelatin concentrations may have been a reflection of aggregation of the gelatin. As their methodology did not identify the cause of turbidity, the

exact nature of the interaction cannot be concluded. If turbidity were merely a result of protein aggregation, it would not be likely for the solution to reach a maximum turbidity and then decrease in turbidity when yet more protein was added to the system.

Woodside et al. (1968) observed that the addition of 0.145 M. sodium chloride inhibited the precipitation in both the neutral and phosphorylated glycogen - gelatin solutions. Since glycogen is neutral, it was not possible to label the inhibitory effects of sodium chloride as being electrostatic in nature. It is more likely that the sodium chloride caused conformational changes in the protein. These conformational changes affected the degree of hydrogen bonding, which in turn led to more stable solutions. Five molar urea and three molar guanidine hydrochloride, neither of which was charged, inhibited precipitation by breaking hydrogen bonds.

Ultra violet absorption spectrum of gelatin and glycogen-gelatin solutions provided evidence of macromolecular interactions between the protein and the polysaccharide. Woodside et al. (1968) also observed dextran - gelatin interactions. No precipitation occurred when dextran was combined with gelatin. Precipitation did result when dextran sulfates were combined with gelatin. This preciptation was dependent on the molecular weight of

the polysaccharide. Disulfide linkages were probably formed between the dextran sulfate and the gelatin. With the large molecular weight polysaccharide, the protein - polysaccharide complex became large enough that it would precipitate. As suggested by So et al. (1969), the interaction between the dextran sulfate and the gelatin may be dependent on configuration of the sugar. A molecular weight of $2 \times 10^6$ resulted in precipitation; one of $5 \times 10^5$ did not. Precipitation in dextran sulfate -gelatin solutions was enhanced by the addition of 0.145 M sodium chloride. This was the only time an increase in protein - polysaccharide precipitation resulting from added sodium chloride has been reported. The sodium chloride appears to have exaggerated disulfide bonding, while at the same time competed for water. Precipitation was inhibited by the addition of 5 M urea or 3 M guanidine hydrochloride. The authors suggested that urea and guanidine hydrochloride dissociated the gelatin into subunits incapable of forming precipitates.

Woodside et al. (1968) found that when mixed with gelatin, an acidic polysaccharide from Cryptococcus laurentii var. flavescens also resulted in precipitation. No explanation was offered. The addition of 0.145 M sodium chloride inhibited precipitation. This would indicate that the precipitate formation was electrostatic in nature.

Like Woodside et al. (1968), So et al. (1969) found neutral polysaccharides could complex with proteins so as to

precipitate. So et al. (1969) observed the effects of many polysaccharides on Concanavalin A. This well conducted study by So et al. (1969) provided evidence that ionic bonding may not be enough to explain precipitation following certain protein - polysaccharide interactions. So et al. hypothesized that hydrogen bonding may play an important role in the formation of protein - polysaccharide precipitates. In one portion of their experimentation, So et al. studied the interaction of Concavalin A and levans; they observed the formation of a precipitate when the two were mixed. So et al. (1969) explained that the interaction between Concanavalin A and polysaccharides was dependent on the configuration of the sugars. They suggested it was the disposition of the hydroxyl groups which determined whether or not the protein - polysaccharide solutions precipitated. Results indicated the precipitate was composed of both protein and polysaccharide. Analysis indicated 70 to 78 % of the total Concanavalin A appeared in the precipitate when precipitation was at a maximum; virtually all of the levan was present in the precipitate. Results indicated that hydrogen bonding was what probably led to precipitation. Since the levans were neutral polysaccharides, electrostatic reactions could not be the cause of precipitation. So et al. (1969) suggested that hydrogen bonding was the mechanism by which the protein and polysaccharide complexed. The authors repeated

experimentation with Concanavalin A and other polysaccharides. The results indicated some polysaccharides such as dextrans and levans resulted in precipitation; others such as D-fructofuranosyl residues served to inhibit precipitation. Unlike Woodside et al. (1968) the authors concluded that the binding sites on Concanvalin A were not specific for various polysaccharides.

## F. GEL FILTRATION

### 1. General Theory

Gel filtration can be used to separate molecules on the basis of molecular size. A porous three dimensional dextran network, known as Sephadex, is poured into a glass column. The network is a gel composed of many glass beads. Different bead sizes can be selected. The size of the bead needed for separation is dependent on the molecular size of the material being passed through the column for fractionation. Once poured, the gel bed is equilibrated with a buffer. As the solute molecules are placed into and pushed through the column, they attempt to equilibrate with the buffer. Large molecules cannot fit into the smaller beads of the gel network; therefore, the larger molecules are flushed right out of the column in what is known as the void volume. Smaller solute molecules penetrate the glass beads. The smaller the molecule, the longer it will remain in the glass beads. Therefore, separation is a result of the relative time it takes the molecules to pass through the column.

Sepharose CL - 6B is a crosslinked agarose gel which is both thermally and chemically stable. The wet bead diameter is 45 to 165 microns. The fractionation ability ranges from a molecular weight of $1 \times 10^{4}$ to $1 \times 10^{6}$. Because it separates over a relatively large range of higher molecular weight molecules, Sepharose CL - 6B would be able to separate

soy protein and xanthan gum. Xanthan gum would likely come off in the void volume; soy protein would remain in the column longer.

The solute elutes from the column due to pressure applied by a pump. As the solute and buffer elute, they are collected in fractions of known volume. The fractions are read on a spectrophotometer to determine absorbance. A reading at 280 nanometers is used to determine protein concentration.

2. Applications

Gel filtration is commonly used for the fractionation of molecules. It is, however, used much less often in the study of protein - polysaccharide interactions. Traditionally, highly viscous solutions do not behave well on the column. A high sample viscosity causes instability and an irregular flow pattern. When eluting viscous materials, the critical value is the viscosity of the sample relative to the eluent or buffer. Because protein - polysaccharide solutions are often quite viscous, relatively few attempts to analyze them via gel filtration have been made.

One experiment where gel filtration was successfully used for the study of protein - polysaccharide interaction was that of Imeson et al. ( 1977 ). Bovine serum albumin ( BSA ) was studied with CMC, dextran, or alginate. It was reported that, in an unheated solution, no interaction

between BSA and any of the polysaccharides occurred. It could have been that a protein - polysaccharide interaction did occur; however, the protein - polysaccharide bond may have been so weak as to be destroyed coming through the column. The researchers reported that when the myoglobin was in solution with CMC, pectate, or alginate there were difficulties with the myoglobin binding to the Sephadex in the column. It was suggested that there was a protein - polysaccharide interaction leading to a conformational change of the protein. Myoglobin alone did not bind to the column. When heated, the elution profiles of the BSA - pectate solutions were changed relative to those of the unheated mixture. The heated protein eluted in smaller volumes than the unheated, native protein. Imeson et al. ( 1977 ) suggested that this indicated a high molecular weight complex was formed between the heated BSA and pectate. This complex would thus inhibit protein - protein aggregation and thereby inhibit precipitation. Because the interactions diminish with increasing ionic strength and increase as the pH decreased form 7 to 5 -- yielding increased positive charge on the protein -- it seems probable that the interactions are electrostatic in nature. The electrostatic nature of the interactions also explains why, at pH 6.0, the interactions are stronger with myoglobin, which possesses a net positive charge at that pH, than with BSA, which carries a net negative charge.

# II. MATERIALS AND METHODS

## A. MATERIALS

### 1. Soy Sauce

Kikkoman Naturally Brewed Soy Sauce was used. It was purchased in a 10 fluid ounce bottle from the local Kroger Supermarket. Once purchased, the soy sauce was stored at room temperature. It was used within two weeks of the purchase date.

### 2. Tamari Sauce

Tamari sauce was purchased through Eats Natural Food Cooperative, Blacksburg, VA. One 25 gallon container was obtained. It was stored at 4 to 6 $^{o}$C until use. As needed, limited quantities were tapped from the 25 gallon drum.

### 3. Soy Protein Isolate

The soy protein isolate (SPI) used throughout the current research was Supro 710 K, a product of Ralston Purina (Product No. C1E-NO27). Tables 1 and 2 supply information on the overall composition and amino acid content of Supro 710K. The sodium content of the soy protein was approximately 1%. At 85% protein, Supro 710K was 5 to 15% lower in protein than a typical soy protein isolate.

The Supro 710K soy protein isolate was stored in moisture resistant containers which were sealed with parafilm and electrical tape. The storage temperature was

-20° C.

4. Whey Protein Concentrate

The whey protein concentrate (WPC) used throughout was Lacprodan 80, a product of Danmark Protein, Worthington, Ohio (Product No. 80-S59). The approximate amino acid content of it is displayed in Table 2.

The Lacprodan 80 was stored in a three layer plastic bag, with drierite between the second and third layer. The storage temperature was approximately 4° C.

5. Xanthan Gum

The xanthan gum used was Keltrol F, a product of Kelco, Division of Merck and Company, Inc.(Product No. 56002A) When not in use, the powdered gum was stored under refrigeration at approximately 4° C.

## B. METHODS

1. Total Nitrogen Determination

Total nitrogen determinations were made on the tamari sauce, the SPI, and the WPC using a modification of the A.O.A.C. ( Thirteenth Edition, 24.027) Kjeldahl procedure. This procedure was modified using zinc and copper as catalysts. A factor of 6.25 was used to make the conversion of total nitrogen to percent protein in the soy samples; for WPC the factor used was 6.38.

Equation (5) was used to calculate total nitrogen.

$$\% \text{ protein} = \frac{(N)(14)(\text{ml HCl titrated})(\text{N conversion factor})(100)}{\text{mg sample analyzed}} \quad (5)$$

The total nitrogen determination was run in triplicate on the tamari sauce, SPI, and WPC.

2. Solution Preparation

a. Xanthan Gum Solutions

A solution of 0.10 % sodium chloride in distilled, deionized water was first made. A 1.0 % xanthan gum solution was then made usin a Lightnin' Mixer ( Series 20 Variable Speed Mixer; 0 to 1750 RPM; Mixing Equipment Company, Inc., Rochester, N. Y. ) with a two inch turbine, three blade propeller. Xanthan gum was added gradually to the 0.1 % sodium chloride solution while being agitated at 500 to 1300 RPM. The RPM's of the Lightnin' mixer were varied so as to keep a constant vortex in the sodium chloride solution to which the gum was being added. Enough xanthan gum was added to make a final solution of 1 % xanthan gum. Blending was continued at approximatey 800 RPM for one hour after all the xanthan gum had been added to insure a homogenous mixture. In the final five minutes of blending, 0.5 ml of sodium azide per 100 milliliters of 1 % xanthan gum solution were added. To ensure optimal hydration, the gum solution was allowed to rest for

twenty-four hours a approximately 4° C before using.

b. Intact Soy Protein Solutions

Soy protein isolate was kept at -20o C until use. The A.O.A.C. Kjeldahl protein determination was run in triplicate on the SPI. Results indicated that it had an average protein content of 85 % on an a wet weight basis. The protein was diluted with distilled, deionized water to a level of 2 % protein. A final concentration of 2 % was selected for two reasons. One reason was that 2 % protein represented a solution which was managable from the standpoint of viscosity. Solutions of greater than 2% were difficult to get into solution; therefore, homogenous solutions were not easily produced. Another reason for having selected a 2 % protein level was that 2 % was a concentration commonly used in many soy drinks and soy-based infant formulas being processed ( Decock, 1974 ). The soy protein solution was made by thoroughly blending 10 grams of SPI with 10 g of distilled, deionized water in a Waring Futura II Dual Range 14 speed blender at a speed setting of 8. The blades of the blender were scraped down to insure a homogenous blend. An additional 431 grams of distilled deionized water was then blended in. This mixture was blended thoroughly for one minute or until homogenous.

c. Hydrolyzed Soy Protein Solutions

To make a hydrolyzed soy protein solution, the above

procedure was followed except that only 392 grams of water was blended with the 50 - 50 blend of soy protein isolate and water. Less water was added here than for the intact solutions in order to allow for the water in the enzyme solution. Blending created a foam. The majority of the foam was allowed to subside. From here, an adaptation of the Hsu et al. ( 1977 ) method of hydrolysis was used. In order to apply Hsu's method the following steps were taken:

i. 100 g of the soy protein solution were brought to 37 °C in a waterbath ( Fisher Shaking Waterbath Model 127 ). The pH was adjusted to 8.0 using 1.5 N NaOH.

ii. An enzyme solution was made by adding 10 g of distilled deionized water to 0.600 g of protease (Bovine Pancrease Crude Type I; Sigma Chemical Company; Product No. 13F-8040). This solution was kept on ice while the pH was adjusted to 8.0 using 0.75 N NaOH .

iii. Immediately after adjusting the pH of the enzyme solution, it was added to the soy protein solution.

iv. This mixture was shaken in a waterbath at 37 °C for fifteen minutes after which the pH dropped to between 6.8 and 7.1.

v. When the pH had dropped to within this range, the

hydrolyzed soy protein solution was immediately removed from the waterbath and chilled in an ice - water bath to stop the enzyme action.

vi. The hydrolyzed soy protein solution was used within 24 hours of being made.

For one portion of the experiment xanthan gum was added to solutions of hydrolyzed soy protein. To assure that the xanthan gum to be combined with the hydrolyzed soy protein solutions would be unaffected by exposure to residual protease the following steps were taken:

i. A 1 % xanthan gum solution was added to distilled deionized water to yield a final solution of 0.25 % xanthan gum.

ii. This solution of 0.25 % xanthan gum was exposed to a modification of the Hsu et al. (1977) enzymatic digestion. The procedure was modified by reducing the time in the waterbath.

iii. The rheological parameters of the enzyme treated, xanthan gum solution were determined (Brookfield RVT Viscometer with UL Adaptor) and compared with those of a 0.25 % xanthan gum solution prepared without the enzyme treatment.

d. Whey Protein Solutions

The Lacprodan 80 was diluted with distilled, deionized water so as to yield a solution of 2 % protein. To complete the dilution, 10 g of the WPC were thoroughly

blended with 10 g of distilled, deionized water, as was done for the soy protein isolate solutions. To this, an additional 386 g of distilled, deionized water was blended. This new mixture was blended for one minute or until homogenous.

3. pH Adjustments

The above soy protein and whey protein solutions were adjusted to different pH's using 1.0 or 2.0 N HCl or 1.0 N NaOH. The soy protein solutions were brought to pH's of 2,3,4,5,7, and 9. The whey protein solutions were brought to pH's of 5 and 7.

4. Protein - Xanthan Gum Solutions

a. Soy Sauce - Xanthan Gum / Tamari Sauce - Xanthan Gum

A 1 % solution fo xanthan gum was added to the soy and tamari sauces. Enough xanthan gum was added to bring the sauces to gum concentrations of 0.05, 0.15, 0.25 % . The mixtures were stirred with a magnetic stir bar for one minute or until no patches of gum were visible. The blended solutions were covered and allowed to sit for 24 hours at 25 $^{o}$C. After the 24 hours, they were visually examined for precipitation. Where no precipitation resulted, the apparent viscosities were determined.

b. Soy Protein - Xanthan Gum

Intact and hydrolyzed soy protein solutions that were adjusted to various pH's were adjusted to various

ionic strengths. Some solutions at each pH were left without sodium chloride. Sodium chloride was added to the remaining soy solutions of pH 5 and 7; they were brought to concentrations of 1,2,3,4,and 5 % sodium chloride. Enough sodium chloride was added to the soy protien solutions at pH 2,3,4,and 9 to bring them to concentrations of 3 % sodium chloride. After the addition of sodium chloride, solutions were stirred with a magnetic stir bar. The above procedure yielded 40 different solutions.

The 1 % solution of xanthan gum was added to each variety of the intact and hydrolyzed soy protein solutions, for final gum concentrations of 0.05, 0.15, and 0.25 %. These mixtures were stirred with a magnetic stir bar for one minute or until no patches of gum were visible. The blended solutions were covered with parafilm and allowed to sit at 25 °C for 24 hours. The soy protein - xanthan gum solutions were generally made in small batches of 30 g each. To every 30 g batch, 0.1 ml of 0.1 % sodium azide was added to prevent bacterial contamination of the samples.

Each variety of solution was made six times.

c. Whey Protein - Xanthan Gum

The whey protein solutions were adjusted to pH's 5 and 7. Some solution of each pH were kept aside to be mixed with xanthan gum. The remainder of the whey protein

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The whey protein solutions were adjusted to pH's 5 and 7. Some solution of each pH were kept aside to be mixed with xanthan gum. The remainder of the whey protein

solutions at pH 5 and 7 were brought to concentrations of 1,2,3,4,and 5 % sodium chloride. After the addition of sodium chloride, the solutions were stirred with a magnetic stir bar.

The 1 % solution of xanthan gum was added to each variety of the whey protein solutions for final gum concentrations of 0.05, 0.15, and 0.25 %. These mixtures were stirred with a magnetic stir bar for one minute or until homogenous. The blended solutions were covered with parafilm and allowed to sit at 25 °C for 24 hours. The whey protein - xanthan gum solutions were made in 30 g batches. Each variety of the whey protein - xanthan gum solutions was made six times.

5. Stability Measurements

a. Background

As stated, the soy protein - xanthan gum solutions were left undisturbed at 25 °C for 24 hours. Those solutions which remained stable were tested for rheological parameters. Stability was determined using two methods, visual and spectrophotometric. The first method was through visual observation, which was later verified through the use of the second method--spectrophotometric measurement of the relative solubility of the protein in the system.

b. Visual Determination

Clear beakers containing the soy protein - xanthan gum solutions were viewed in front of a 100 watt spotlight. Solutions with signs of flocculence, either on the surface or throughout the solution, or with precipitation, or with both were classified as unstable. It should be noted that solutions with negligible precipitate, meaning one or two tiny specks, were accepted as stable as long as no floc existed. All other solutions were classified as stable. The same procedure was followed when whey protein - xanthan gum solutions were studied.

After this visual examination, a small amount of each solution was carefully plated on a microscope slide. A photograph of each solution was taken ( Nikon F-4 35 millimeter camera ). Light was provided from a light box beneath the slide. The film used was Kodak Black and White Technical Pan (lot number CAT 129 7563). Film was developed by the Learning Resources Division of Virginia Polytechnic and State University.

c. Spectrophotometric Determination

After visual observation, solutions were poured from beakers into screw cap test tubes and centrifuged at 3,020 g. for thirty minutes. The supernatant was drawn off and diluted appropriately so it could be read on a spectrophotometer (Bausch and Lomb 2000). All samples

were read at 280 nanometers using distilled, deionized water as a blank. A correlation coefficient of 0.99 indicates the method is capable of accurately determining protein concentration. Controls were 0.05, 0.15, and 0.25 % xanthan gum in water, as well as solutions of intact and hydrolyzed soy protein without gum added. All controls were centrifuged according to the above procedure. The appropriate absorption value of the xanthan gum - in - water control was subtracted from the soy protein - xanthan gum absorbance readings. The 2 % intact and hydrolyzed soy protein solutions were centrifuged in a similar manner. The absorbance readings of the 2 % intact and hydrolyzed soy protein without gum added were set equivalent to 100 % soluble protein and were used as reference controls for solutions of intact soy - xanthan gum and hydrolyzed soy - xanthan gum, respectively. Thus, the proportion of soluble protein in the sample solutions was calculated relative to the absorbance of the appropriate control.

Results of the objective stability measurements were compared to those of the visual stability determination.

The above procedures were repeated for whey protein - xanthan gum solutions.

6. Rheology

The rheological parameters of stable soy protein - xanthan gum systems were measured using the same technique throughout the study. Soy protein - xanthan gum solutions were prepared by adding a 1 % presolution of xanthan gum to the 2 % soy protein solutions at the ranges of pH and sodium chloride concentrations noted earlier.

The apparent viscosities of each visually stable solution was determined at 2.5,5.0,10.0,20.0,50.0, and 100 RPM at 25 $^{o}$C ( Brookfield RVT Rotoviscometer with UL Adaptor ). The readings were taken only after the viscometer had made ten complete revolutions. The readings indicated on the viscometer were multiplied by the appropriate factors, provided by Brookfield, to yield apparent viscosity measurements in centipoise units. All the apparent viscosity data was plotted on log-log graph paper with the log of the shear rate in reciprical seconds on the horizontal axis and the log of the apparent viscosity on the vertical axis. The slope and the y-intercept of each graph was calculated using the straight line equation:

$$y = mx + b \qquad (6)$$

The flow behavior index and consistency coefficient were calculated for each solution tested using the Power - Law Model.

The Power Law Relationship is described by the equation:

$$\lambda = m\gamma^{n}$$

where $\lambda$ = shear stress ( dynes/cm$^{2}$ )

$m$ = consistency coefficient (cps)

$\gamma$ = shear rate (sec$^{-1}$ )

$n$ = flow behavior index

It can be shown through derivation of the Power Law Model that:

$$n = \text{slope} + 1$$

Plots of the soy protein - xanthan gum solutions were compared with similar xanthan gum - water controls. For example, a solution of 0.25 % xanthan gum in 2 % soy protein was compared to a solution of 0.25 % xanthan gum in water. This method allowed for detection of possible viscosity synergism. The apparent viscosities of the intact and hydrolyzed soy protein were accounted for in the comparison. Statistics were performed comparing flow behaviors and consistency coefficients of the soy protein - xanthan gum solutions to those of the appropriate controls.

7. Gel Filtration

A gel filtration column ( American Scientific Products; column C4660-11; length = 400mm; diameter = 15mm ) was packed with a gel ( Sepharose CL - 6B; Pharmacia Fine Chemicals ). A pump ( LKB Vario II Pump ) with a speed of 0.8888 ml per minute was used to pack it. The

column was allowed to equilibrated with a 0.10 molar Tris - HCl buffer of pH 7.0. The buffer was deaerated prior to pumping through the column in order to prevent the development of air bubbles in the gel. The integrity of the column was checked using blue dye. An even flow of the blue color down the column indicated a well - packed column.

A 1.0 ml sample of filtered soy protein solution was gravity fed into the packed column. The sample in the column was followed by the addition of 2.0 ml of buffer that was also gravity fed. A pump speed of 0.160 ml per minute was used to continue the fractionation. At least 200 ml of solution was collected. Each collection tube contained 2.5 ml of sample. The collected fractions were read on a spectrophotometer ( Bausch & Lomb 2000 ) at 280 nanometers. Elution profiles were drawn. This was done in duplicate or until a standard, reproducible profile was obtained.

The above procedure for sample application was attempted using a 2 % intact soy protein - 0.25 % xanthan gum solution. Recovery of the proteins was determined on the spectrophotometer at 280 nanometers. Recovery of the xanthan gum was determined via the phenol - sulfuric acid reaction developed by Erikson and Richardson ( 1957 ).

# III. RESULTS AND DISCUSSION

## Introduction

The purpose of this study was to determine the effects of protein type, sodium chloride concentration, xanthan gum concentration, and pH on the reaction between soy protein and xanthan gum. It was known that xanthan gum causes most proteins to precipitate. However, preliminary investigation indicated soy protein was unique in that it remained stable in the presence of xanthan gum.

Throughout this discussion, soy protein will be referred to as intact or hydrolyzed. Intact soy refers to Ralston Purina Supro 710K as purchased. Hydrolyzed soy refers to Ralston Purina Supro 710K that has been exposed to proteolytic digestion.

The results of this study are discussed in five sections: (1) preliminary findings; (2) stability of protein-xanthan gum solutions; (3) rheological parameters of the soy protein - xanthan gum solutions; (4) solubility of the protein within protein - xanthan gum solutions; (5) chromotography. Sections one, three, and five deal specifically with soy protein - xanthan gum solutions; sections two and four also include whey protein - xanthan gum solutions.

## A. PRELIMINARY FINDINGS

Preliminary work included comparison of the rheological parameters of soy sauce - xanthan gum solutions to those of tamari sauce - xanthan gum solutions. It also included work to determine the feasibility of using the Hsu et al. (1977) method for enzymatic hydrolysis on soy protein isolate. The results and discussion is found below.

### 1. Rheological Parameters of Soy Sauce - and Tamari Sauce - Xanthan Gum Solutions

When mixed with xanthan gum, both the soy sauce and tamari sauce formed stable, homogenous solutions. These results agreed with earlier findings ( personal communication, Saltmarch, 1982 ) which indicated that soy sauce - xanthan gum solutions were stable.

Figures 11 and 12 illustrate rheograms of soy sauce and tamari sauce solutions at gum concentrations of 0.15% and 0.25%, respectively. An appropriate control of xanthan gum in water is included for comparison. It should be noted that while both sauces exhibited a viscosity synergism, it was, in both cases, greater for the tamari sauce solution. This could be attributed to the fact that the tamari sauce contained more protein than did the soy sauce. It could also be attributed to the fact that tamari sauce is comprised of only soy protein, whereas soy sauce is comprised of both soy and wheat protein.

The rheological parameters of these solutions were

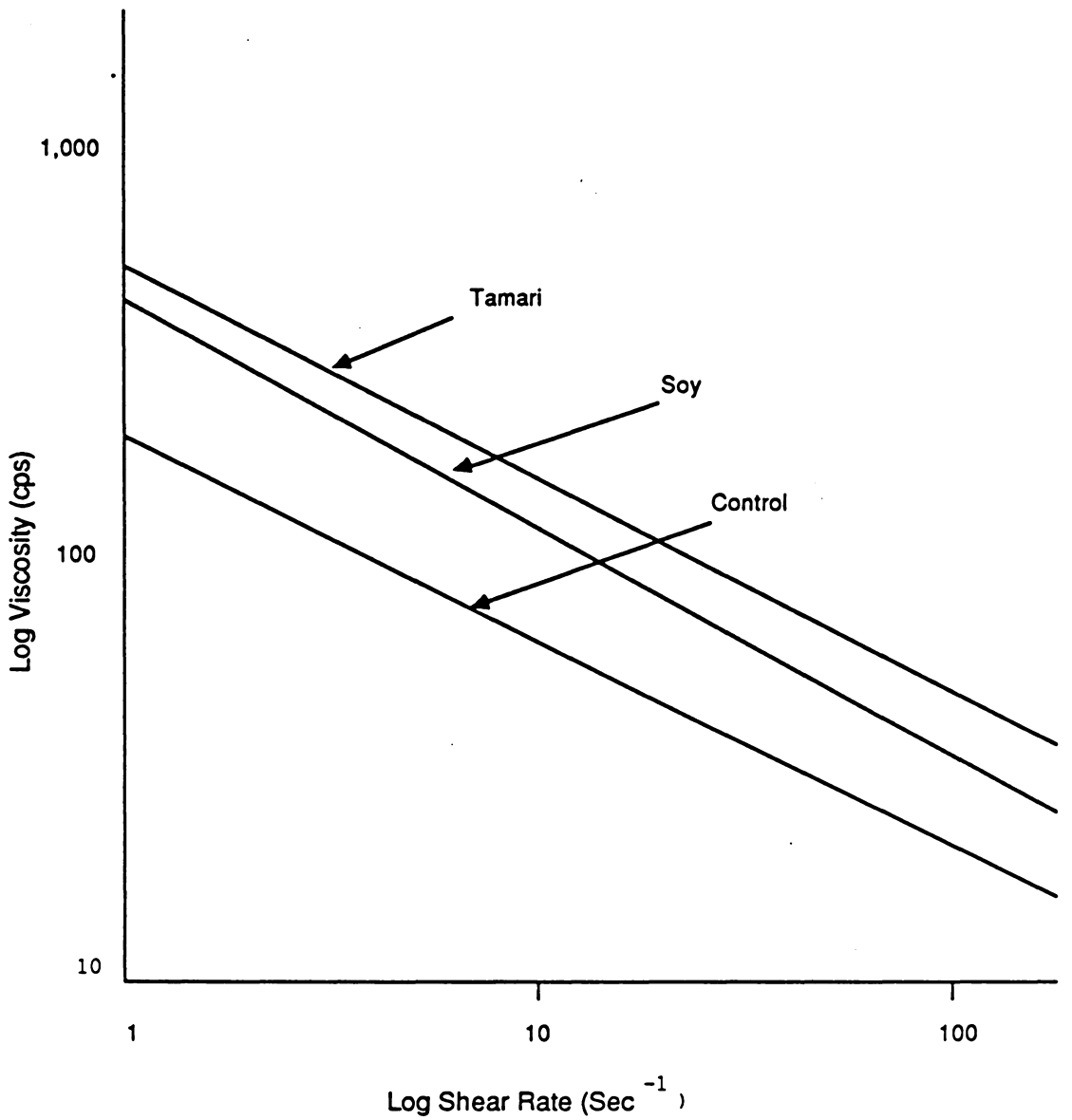


**Figure 11. Apparent Viscosities of Soy Sauce and Tamari Sauce with 0.15% Xanthan Gum**

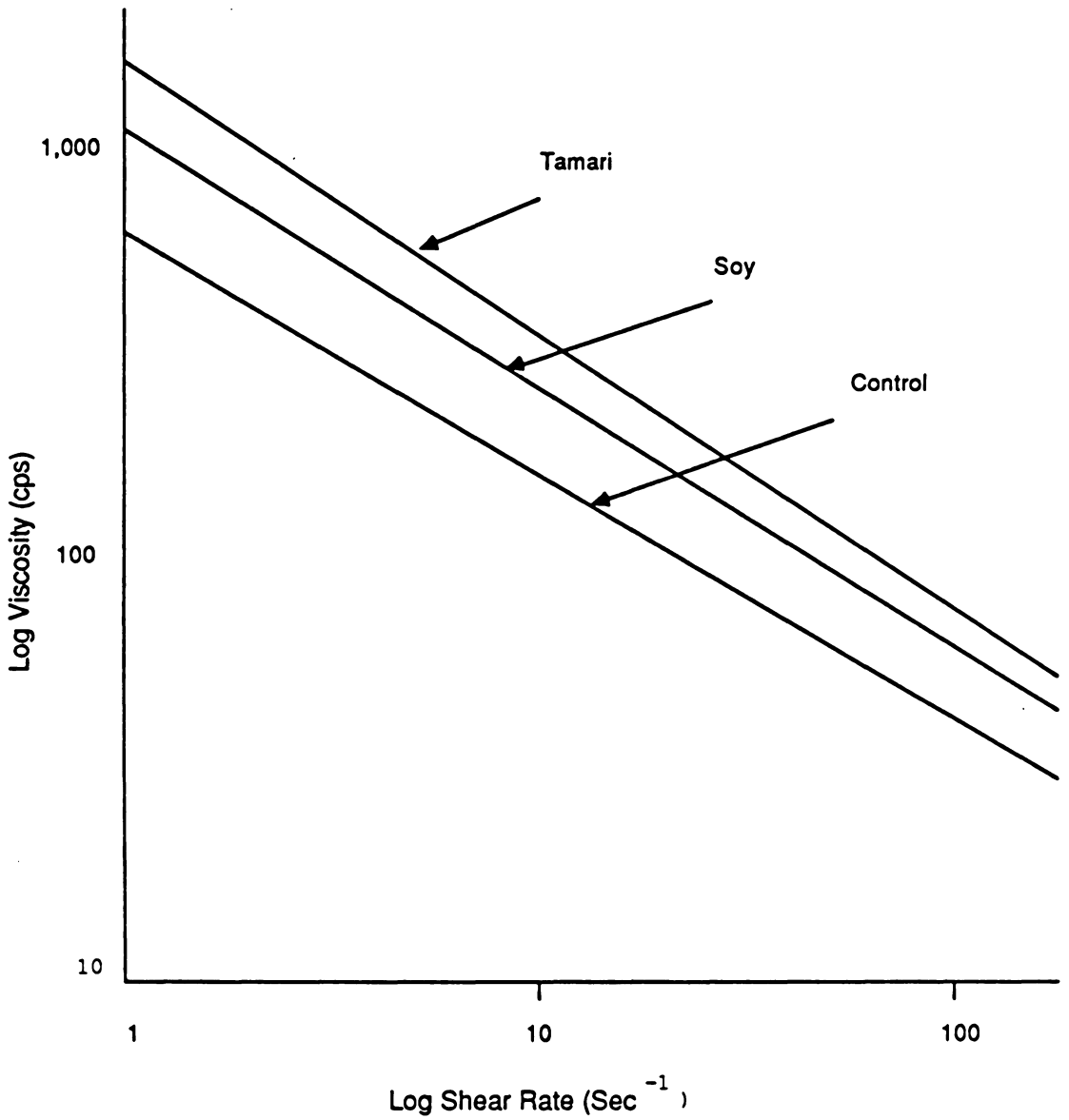


**Figure 12. Apparent Viscosities of Soy Sauce and Tamari Sauce with 0.25% Xanthan Gum**

calculated and are listed in Table 4. It can be seen that the flow behavior indices, n, of the control, soy sauce, and tamari sauce solutions were similar within either level of xanthan gum. This similarity is confirmed in Figures 11 and 12 where the lines are nearly parallel. At both 0.15% and 0.25% xanthan gum, the tamari sauce solutions were only slightly less pseudoplastic than were the controls or the soy sauce solutions.

From Table 4 it can be seen that solutions with 0.25% xanthan gum were more pseudoplastic than similar solutions with 0.15% xanthan gum. For example, tamari sauce - 0.25% xanthan gum solutions had a flow behavior index, n, of 0.338 while tamari sauce - 0.15% xanthan gum solutions had an index of 0.439. The smaller the flow behavior index, the more pseudoplastic the solution.

Comparing consistency coefficients, m, of solutions with 0.15% xanthan gum, it can be seen that for the soy sauce solution the consistency coefficient was 436 cps. For the control it was 190 cps. This is a difference of 246 cps. The tamari sauce - 0.15% xanthan gum solution had a consistency coefficient of 495 cps, or 305 cps. above that of the control. Comparison indicated the viscosity synergism observed in tamari sauce - 0.15% xanthan gum solutions was 59 cps. greater than in soy sauce - 0.15% xanthan gum solutions. The viscosity synergism was greater for the tamari sauce -

TABLE 4. Flow Behavior Index (n) and Consistency Coefficients (m) of Solutions in Which 0.15% or 0.25% Xanthan Gum was Blended with Water, Soy Sauce, or Tamari Sauce

| | 0.15% Gum | | | 0.25% Gum | | | |
|---|---|---|---|---|---|---|---|
| Solution | n | m | Above Control* | n | m | Above 0.15%** | Above Control* |
| Control | 0.424 | 190 | - | 0.344 | 685 | 27.7 | - |
| Soy | 0.409 | 436 | 246 | 0.309 | 1262 | 34.5 | 577 |
| Tamari | 0.439 | 495 | 305 | 0.338 | 1300 | 38.1 | 615 |

* Centipoise above the consistency coefficient, m, of the control

** Percentage above the consistency coefficient of the similar solution with 0.15% xanthan gum

xanthan gum solutions than for the soy sauce - xanthan gum solutions.

Results from solutions with 0.25% xanthan gum indicate a similar trend. The consistency coefficient of the soy sauce - 0.25% xanthan gum solution was 1262. For the control, 0.25% xanthan gum in water, the consistency coefficient was 685 cps. The difference between the consistency coefficient of the soy sauce - 0.25% xanthan gum solution and the control was 577 cps. The difference between the consistency coefficient of tamari sauce - 0.25% xanthan gum solutions and the control was 615 cps. The tamari sauce - 0.25% xanthan gum solution had a consistency coefficient that was 78 cps. greater than that of the soy sauce - 0.25% xanthan gum solution.

There are two characteristics of tamari sauce that might explain why the tamari sauce - xanthan gum solutions were more pseudoplastic and had larger consistency coefficients than similar soy sauce - xanthan gum solutions. Tamari sauce is 14.5% protein, whereas the soy sauce was only 10% protein. It is proposed that the higher concentration of protein in the tamari sauce provided more reactive sites with which the xanthan gum could react. The tamari sauce contains only soy protein; soy sauce contains both soy and wheat proteins. It is hypothesized that the xanthan gum could react more readily with soy protein than with wheat protein.

The consistency coefficient of the control at 0.25%

xanthan gum was 3.6 times that of the control at 0.15% xanthan gum. The consistency coefficient of the soy sauce- 0.25% xanthan gum solutions was 2.35 times greater than that of the soy sauce- 0.15% xanthan gum solutions. The consistency coefficient of the tamari sauce - 0.25% xanthan gum solution was only 2.02 times greater than that of the tamari sauce - 0.15% xanthan gum solutions.

It is hypothesized that there exists a dynamic balance between gel formation and precipitation in the soy sauce and tamari sauce - xanthan gum solutions. At a certain protein concentration the gel formation would be at a maximum. If that given level of protein were exceeded, it is proposed that there would be more protein - xanthan gum interaction, which would result in some precipitation. More precipitation would result in less soluble aggregate formation and hence a decreased m value. In the tamari sauce - 0.25% solutions it is possible that the protein - xanthan gum interaction exceeded the maximum and thus resulted in some precipitation and, consequently, slightly less of an increase in the consistency coefficient than might have been expected.

It could also be possible that there is a dynamic balance between gel formation and precipitation in protein - xanthan gum solutions. It could be that in the solutions with 0.15% xanthan gum, the gel formation was at a maximum. Upon increasing the concentration of xanthan gum to 0.25%, it

could be that the balance between gelation and precipitation of the protein moved away from gel formation and more towards precipitation. An increase in precipitate, and thus a decrease in gel formation, could result in a decreased consistency coefficient. This balance between gel formation and precipitation is similar in concept to the mechanism proposed by Wolf and Tamura (1969), which proposed that soy proteins form soluble aggregates that later convert to insoluble precipitates.

## 2. Enzymatic Hydrolysis of Soy Protein Isolate

The first objective of this portion of the study was to determine whether protease could be used in place of the multienzyme system suggested by Hsu et al. (1977). Results indicated that, indeed, protease could be used successfully. Hsu et al. (1977) indicated that hydrolyzed soy protein isolate should have a pH of 6.9 following a ten minute incubation with a multienzyme system. As can be seen in Figure 13, protease alone effected a similar drop in pH. It was noted that the pH drop effected by either enzyme system varied by as much as 0.3 following ten minutes of incubation. While this variation is small, it does indicate that hydrolysis may be only partial.

A further indication that the ten minute incubation did not affect complete hydrolysis is that, throughout refrigerated storage, the pH of the hydrolyzed soy solutions continued to drop gradually. This is illustrated in Figure 14. It should be noted that Figure 14 illustrates the change in solutions which where initially hydrolyzed to different degrees. Because of the gradual decrease in pH over time, all solutions of hydrolyzed soy were used within twenty - four hours of digestion and at a set time after digestion to minimize differences in the degree of hydrolysis.

Another objective of this portion of the study was to

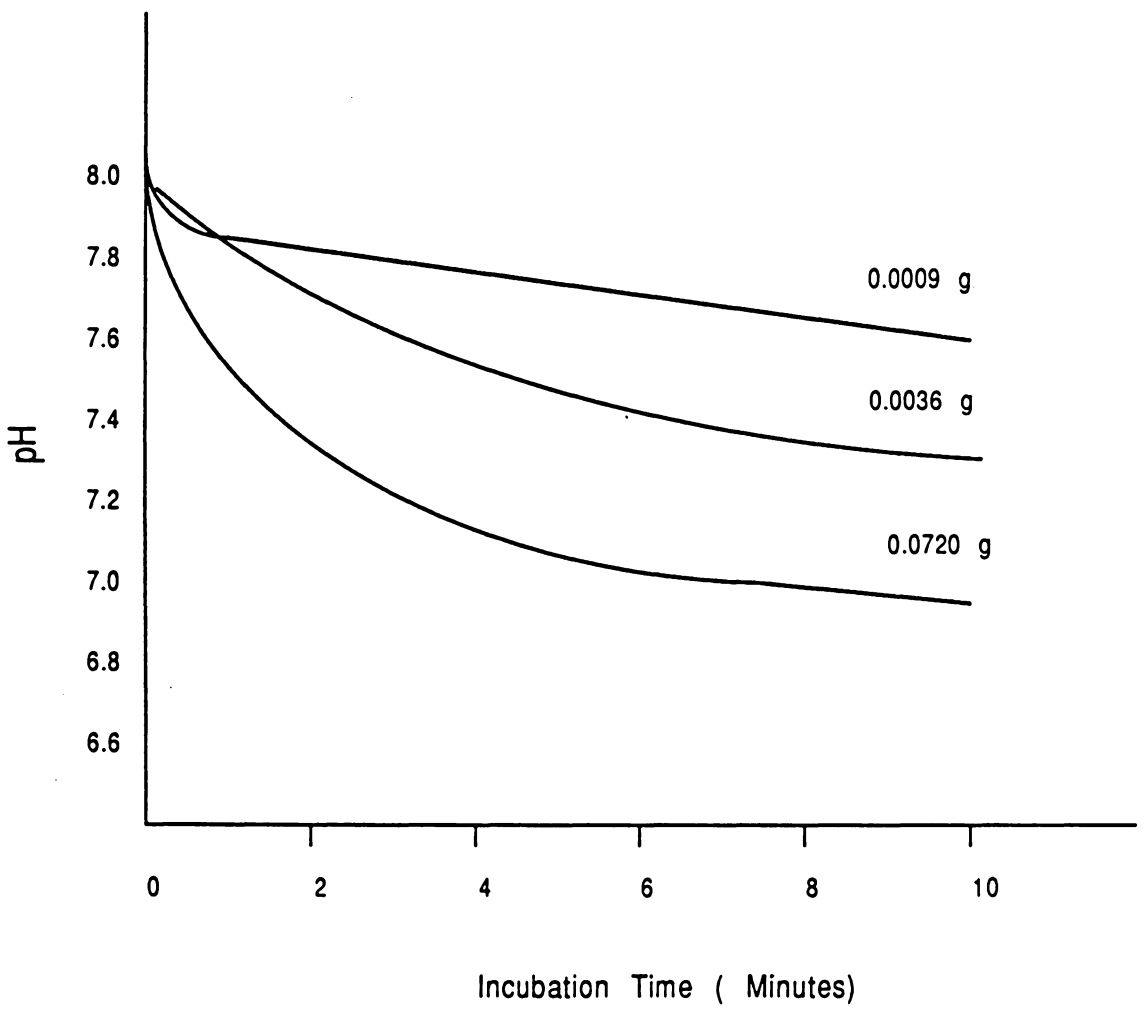


**Figure 13: pH vs. Time Curves Obtained by Incubation of Soy Protein Isolate with Various Amounts of Protease: An Index of Protein Hydrolysis**

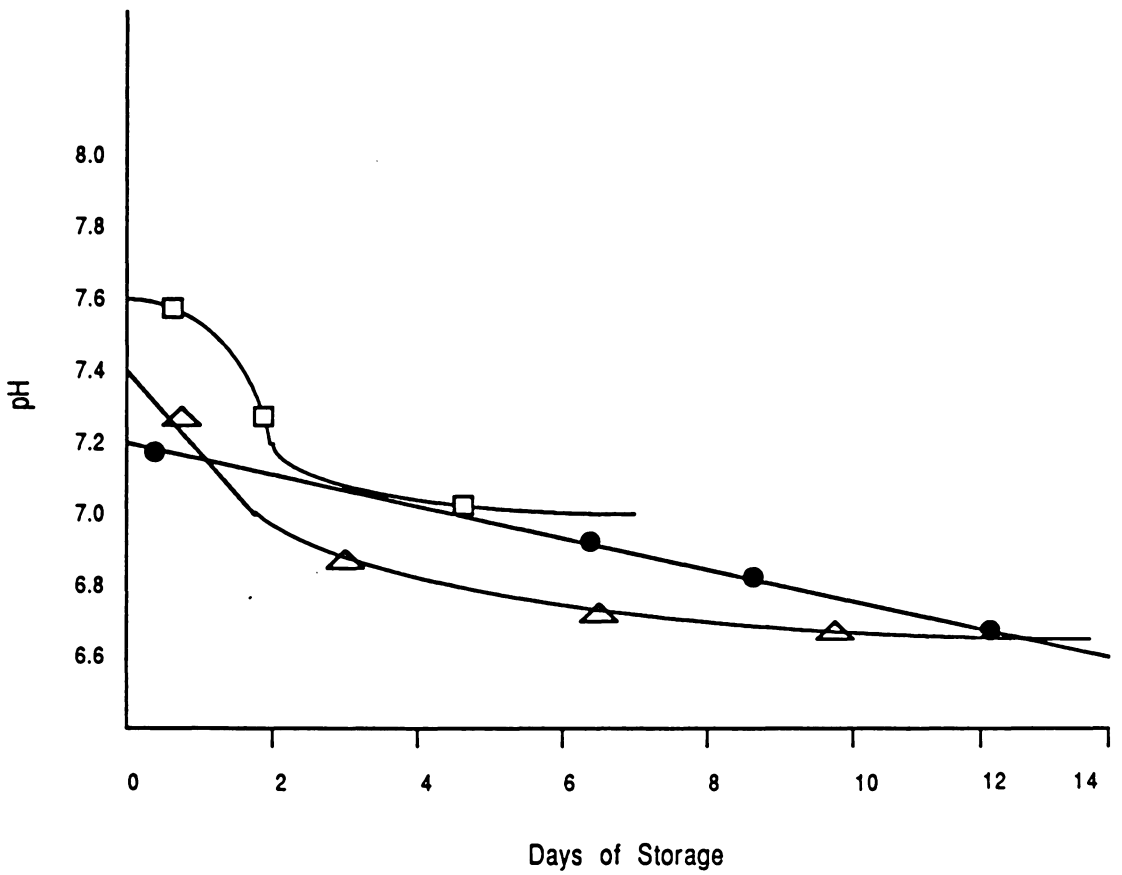


**Figure 14. pH vs. Time Curves Obtained by Refrigerated Storage of Several Hydrolyzed Soy Protein Isolates, Starting at Different pH's**

determine the extent to which the degree of hydrolysis could be controlled. The level of hydrolysis could be controlled somewhat by altering the levels of enzyme used in the digestion. Figure 13 illustrates that using protease at 0.05 times the level recommended by Hsu et al. (1977), resulted in less complete hydrolysis of the protein, as indicated by the reduced drop in pH. However, large differences in enzyme level resulted in relatively small differences in pH following ten minutes of incubation. Because the differences in pH were so small, the pH's of even the hydrolyzed soy solutions had to be adjusted using hydrochloric acid and sodium hydroxide whenever a pH below 6.5 or above 7.8 was desired.

Analysis indicated that xanthan gum was unaffected by exposure to the protease used in the Hsu et al. (1977) protein digestibility method. Viscosity measurements of the xanthan gum solutions before and after exposure to the protease indicated no change in viscosity.

## B. Stablility of Soy Protein and Whey Protein Solutions with Xanthan Gum

### 1. Soy Protein

#### a.Solutions at pH 7

According to Wolf and Tamura (1969) and Shen (1981) soy proteins at pH 7 are only partially soluble. Wolf and Tamura (1969) proposed a mechanism whereby soy proteins first form soluble aggregates; some of these soluble aggregates go on to form insoluble precipitates. Throughout this research, it was observed that, indeed ,soy protein at pH 7 did exhibit some precipitation. However, it was noted that the addition of xanthan gum to the soy protein resulted in greater precipitation than was observed in solutions of soy protein without xanthan gum. Through this research it was observed that the addition of varying levels of sodium chloride and xanthan gum effected solution stability.

Tables 5 and 6 indicate that intact and hydrolyzed soy protein - xanthan gum solutions at pH 7 with 0% added sodium chloride were not stable. All showed signs of gross flocculence and precipitation. Intact soy protein solutions were opaque white. As the intact soy protein - xanthan gum solutions were allowed to sit, a white layer of precipitate settled at the bottom of the beaker and the effluent remained milky white. In contrast to the solutions of intact soy, those with hydrolyzed soy were relatively clear, and had a yellowish

Table 5

## PERCENTAGE OF STABLE SOY PROTEIN - XANTHAN GUM SOLUTIONS

INTACT SOY pH 7

| GUM (%) | n | SODIUM CHLORIDE CONCENTRATION (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| 0.05 | 6 | 0 | 0 | 0 | 17 | 17 | 17 |
| 0.15 | 6 | 0 | 33 | 17 | 50 | 100 | 83 |
| 0.25 | 6 | 0 | 17 | 50 | 100 | 100 | 100 |

n = six repetitions per variable

Table 6

## PERCENTAGE OF STABLE SOY PROTEIN - XANTHAN GUM SOLUTIONS

HYDROLYZED SOY pH 7

| GUM (%) | n | SODIUM CHLORIDE CONCENTRATION (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| 0.05 | 6 | 0 | 0 | 33 | 67 | 17 | 33 |
| 0.15 | 6 | 0 | 17 | 67 | 100 | 100 | 100 |
| 0.25 | 6 | 0 | 17 | 50 | 67 | 67 | 67 |

n = six repetitions per variable

cast. The precipitate in the hydrolyzed soy solutions appeared as small white specks settled at the bottom. It was also common to see flocculence in hydrolyzed soy protein xanthan gum solutions.

As seen in Tables 5 and 6, intact and hydrolyzed soy protein - xanthan gum solutions at pH 7 with 1% added sodium chloride generally were not stable. These results are interesting in light of Shen's theory of protein solubility. Shen (1981) explained that increasing salt concentrations beyond 0.1 molar results in increasing the amount of associated soy protein. The associated soy proteins have fewer exposed hydrophobic groups (Shen, 1981). The reduction in exposed hydrophobic groups results in a soy protein molecule that is more unfolded. The expanded structure of the unfolded soy protein made it available for interaction with available xanthan gum. As the soy protein- xanthan gum solutions with 1% added sodium chloride were 0.17 molar, it would, according to Shen, be expected that they would have been more fully unfolded and hence more stable. However, it appears that as the soy protein unfolded it formed insoluble complexes with the xanthan gum.

It is hypothesized that the interaction between unfolded soy protein and xanthan gum is similar to the interaction between xanthan gum and galactomannans (Pettitt, 1982). Xanthan gum is able to form hydrogen bonds with the electronegative regions of the soy protein. It is reasonable

to believe that the unfolded, segments of soy protein complexe with xanthan gum in its native helical conformation, to produc junction zones of varying complexity. As explained by Pettitt (1982), the degree of complexity would depend on the degree to which the soy protein was branched.

As noted by Kelco (1975), the helical structure of xanthan gum is unusually stable. The solubility of xanthan gum is relatively unaffected by pH or salt concentration. Therefore, if xanthan gum were present in sufficient concentration, the soy proteins that were bound to the xanthan gum structure would be less affected in terms of solubility than similar soy proteins which were not integrated into the xanthan gum structure. It can be seen in Table 5 that 33% of the intact soy protein - 0.15% xanthan gum solutions and 17% of the intact soy protein - 0.25% xanthan gum solutions with 1% added sodium chloride were stable. At 0.15 and 0.25%, the xanthan gum exerted a large enough charge to affect the solution environment. The charged groups on the xanthan gum resulted in repulsive forces strong enough to stablilize the soy protein - 0.15 and 0.25% xanthan gum solutions.

Table 6 indicates that 17% of the hydrolyzed soy protein solutions at pH 7 with 1% added sodium chloride and 0.15 or 0.25% xanthan gum were stable. These few stable solutions are again attributable to the effect of the xanthan gum on

the solution environment. The charged groups of the xanthan gum created repulsive forces, which prevented some of the soy protein from aggregating. Proteins that did not aggregate were available to form soluble complexes with xanthan gum. This agrees with the results observed by Hidalgo and Hansen (1969) and by Ganz (1974).

Table 5 shows the effect of the addition of 3% added sodium chloride on intact soy protein - xanthan gum solutions. It can be seen that 50% of the soy protein - 0.25% xanthan gum solutions at pH 7 and 3% added sodium chloride were stable. This is the result that would be expected according to Shen's theory (1981). At 3% added sodium chloride, the structure of soy protein isolate was less compact than at the lower sodium chloride concentrations, thus allowing for more reaction with xanthan gum.

From Tables 5 and 6 it can be seen that sodium chloride concentrations of 2 to 5% resulted in increased stability of the soy protein - xanthan gum solutions, when xanthan gum was at concentrations of 0.15 or 0.25%. At the lower xanthan gum concentration of 0.05%, there was not enough gum available to bind the unfolded, unbranched segments of the soy protein. As the xanthan gum concentration increased to 0.15%, there was enough gum available to the soy protein to stabilize it.

Table 5 summarizes the effects of increasing concentrations of added sodium chloride on intact soy protein

- xanthan gum solutions. For solutions with 0.25% xanthan gum, maximum stability was observed at 3, 4, and 5% sodium chloride. It is interesting to note in Table 5 that 4% sodium chloride was necessary to obtain maximum stability when only 0.15% xanthan gum was added to the intact soy solutions. As less charge is contributed from 0.15% xanthan gum than from 0.25% xanthan gum, the additional charge from 4% sodium chloride was probably needed to unfold the soy protein and thereby form and stabilize the soy protein - xanthan gum complexes. Hidalgo and Hansen (1969) and Ganz (1974) also reported an increase in solubility of CMC - protein solutions when the CMC was added in excess. Table 5 supports their finding. While they did not offer a hypothesis for the increased stability at higher CMC concentrations, it is perhaps that the excess CMC altered the ionic environment of the solution. Another possible explanation for increased solubility as the gum concentration was increased is the concept of a balance between gel formation and precipitation. It is suggested that at a given ratio of gum to protein, gel formation reaches a maximum. Upon exceeding this given ratio of gum to protein, there would be more protein - gum interaction, which would in turn result in precipitation.

In addition to causing the unfolding of the soy protein, the higher concentrations of sodium chloride created charge repulsion which would prevent the aggregation of the soy

protein - xanthan gum complexes which had formed.

Table 6 summarizes the stability results of hydrolyzed soy protein - xanthan gum solutions. It should be noted that 67% and 50% of the hydrolyzed soy protein solutions at pH 7 with 2% added sodium chloride were stable when blended with 0.15 and 0.25% xanthan gum, respectively. Shen (1981) concluded that more sodium chloride was needed to salt - in hydrolyzed soy protein than to salt - in intact soy protein. Shen stated that 0.1 molar sodium chloride would salt - in intact soy, while 0.2 molar sodium chloride was needed to salt - in hydrolyzed soy. According to this, it was expected that more sodium chloride would be required to stabilize hydrolyzed soy than to stabilize intact soy. As stated by Shen (1981), sodium chloride concentrations above 0.2 molar increased the solubility of hydrolyzed soy protein. Two percent added sodium chloride caused the hydrolyzed soy protein aggregates to unfold, thereby making the soy protein structure favorable for interaction with xanthan gum. The unfolded, unbranched sections of the soy protein hydrogen bond with the xanthan gum. Because the hydrolyzed soy proteins were smaller and less branched than the intact soy proteins, they may have bound to xanthan gum more readily than did the intact soy proteins.

A comparison of Tables 5 and 6 indicates that less sodium chloride was required to stablilize hydrolyzed soy protein - xanthan gum solutions than intact soy protein - xanthan gum solutions. This is the opposite of what was

predicted by Shen (1981). Over 50% of the hydrolyzed soy protein - xanthan gum solutions were stabilized with 2% sodium chloride, whereas 3% sodium chloride was required before the majority of the intact soy - xanthan gum solutions were stabilized.

The fact that less sodium chloride stabilized solutions of hydrolyzed soy protein - xanthan gum could be explained based upon protein structure. Molecules of hydrolyzed soy are smaller than those of intact soy. A partial unfolding of the hydrolyzed soy protein aggregates left protein segments which fit into the xanthan gum structure more easily than did the larger segments of the intact soy.

As seen in Table 6, 3 to 5% sodium chloride resulted in maximum stability of the hydrolyzed soy protein - 0.15% xanthan gum solutions at pH 7. Hydrolyzed soy protein - 0.25% xanthan gum solutions were less stable than similar solutions with 0.15% xanthan gum. In this case, an excess of the xanthan gum did not improve stability. This is contrary to what was observed by Hidalgo and Hansen (1969) for intact protein - xanthan gum solutions. At 3 to 5% added sodium chloride, 67% of the hydrolyzed soy solutions with 0.25% xanthan gum were stable; whereas, with 0.15% xanthan gum the same solutions were 100% stable. The loss of stability at the higher gum concentrations is similar to what was observed by Heubner and Wall (1979) in their study of winter wheat with

the acidic polysaccharide, Arthrobacter viscosus. Excessive hydrogen bonding of the hydrolyzed soy protein - 0.25% xanthan gum solutions at pH 7 with 3 to 5% added sodium chloride resulted in protein - xanthan gum complexes that precipitated due to their large molecular weight.

b. Solutions at pH 5

Stability results for intact and hydrolyzed soy protein-xanthan gum solutions at pH 5 are summarized in Tables 7 and 8. From Table 7 it can be seen that the only intact soy protein - xanthan gum solutions with greater than 50% stability contained 0.25% xanthan gum. pH 5 approaches the isoelectric point of soy protein, which is 4.2. At pH 5 there is relatively little net charge on the soy protein. Therefore, at pH 5, soy protein is compact and inclined to precipitate. It is interesting to note that at pH 5, 50% of the intact soy - 0.25% xanthan gum solutions with no added sodium chloride were stable. This indicates that the xanthan gum functioned to salt - in the soy protein. The charged residues of xanthan gum had an effect similar to that of greater than 0.1 molar sodium chloride on soy protein. Table 8 indicates that hydrolyzed soy protein - 0.25% xanthan gum solutions at pH 5 with no added sodium chloride were also stable in 50% of the cases. Xanthan gum had a salting - in effect on the hydrolyzed soy protein. The ability of xanthan gum to salt - in the soy protein appeared to be due to the charged residues on the gum.

Table 7 summarizes the effects of adding up to 5%

Table 7

## PERCENTAGE OF STABLE SOY PROTEIN - XANTHAN GUM SOLUTIONS

INTACT SOY pH 5

| GUM (%) | n | SODIUM CHLORIDE CONCENTRATION (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| 0.05 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.15 | 6 | 33 | 17 | 17 | 17 | 17 | 17 |
| 0.25 | 6 | 50 | 100 | 100 | 83 | 100 | 67 |

n = six repetitions per variable

Table 8

## PERCENTAGE OF STABLE SOY PROTEIN - XANTHAN GUM SOLUTIONS

HYDROLYZED SOY pH 5

| GUM (%) | n | SODIUM CHLORIDE CONCENTRATION (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| 0.05 | 6 | 33 | 33 | 17 | 33 | 17 | 0 |
| 0.15 | 6 | 50 | 67 | 67 | 50 | 17 | 33 |
| 0.25 | 6 | 50 | 83 | 67 | 67 | 50 | 33 |

n = six repetitions per variable

sodium chloride to intact soy protein - xanthan gum solutions at pH 5. It can be seen that together with 0.25% xanthan gum as little as 1% added sodium chloride unfolded the soy protein such that it could form a soluble complex with the xanthan gum. Stability of the intact soy protein - 0.25% xanthan gum solutions at pH 5 with 5% sodium chloride dropped to 67%. It is proposed that excessive hydrogen bonding between the soy protein and xanthan gum resulted in large insoluble complexes.

Results of hydrolyzed soy protein - xanthan gum solutions at pH 5 are given in Table 8. It can be seen that of the hydrolyzed soy protein - xanthan gum - sodium chloride variations studied, none were stable 100% of the time. It is interesting to note that most of the hydrolyzed soy - 0.15% and 0.25% xanthan gum solutions at pH 5 were at least 50% stable. Because the hydrolyzed soy proteins were smaller in size than intact soy proteins, the hydrolyzed soy was able to bind to the available xanthan gum. It is proposed that excessive binding of the protein to the gum resulted in less than 100% of the hydrolyzed soy protein - xanthan gum solutions being stable.

A comparison of Tables 5 and 7 indicates fewer solutions of intact soy at pH 5 were stable than were intact soy solutions at pH 7 when mixed with 0.15% xanthan gum. Soy molecules in solutions at pH 5 were more compact than those

at pH 7; thus soy protein at pH 5 would require more unfolding before it could complex with xanthan gum. Comparison of the pH 5 and pH 7 intact soy solutions indicated that with 0.25% xanthan gum, the pH 5 solutions were stable more frequently than those at pH 7. This indicates that at the lower pH, xanthan gum exerted more of an ionic effect on the solution environment.

A comparison of Tables 6 and 8 indicated results of hydrolyzed soy protein - xanthan gum solutions at pH's 7 and 5, respectively. As with the intact soy solutions at pH 7 and 5, hydrolyzed soy protein solutions at pH 5 were generally less stable than those at pH 7 when combined with 0.15% xanthan gum. Ganz (1974) reported similar findings. Ganz found that reducing the pH of stable CMC - soy solutions resulted in precipitation. Ganz hypothesized that at the lower pH, the carboxyl groups of the CMC combined more with the positively charged hydrogen ions in solution than with the positively charged protein residues. Perhaps a similar situation exists for the xanthan gum - soy protein solutions studied. At 0.25 %, the xanthan gum was available in large enough quantities that all the carboxyl groups were not bound by the positively charged hydrogen ions in solution. Tables 6 and 8 indicate less of a pH effect when the gum was at 0.25%.

2. Whey Protein

Tables 9 and 10 summarize stability results of intact

whey protein - xanthan gum solutions at pH 7 and 5. It can be seen that no intact whey protein - 0.05% xanthan gum solutions were stable. It should also be noted that no intact whey protein - xanthan gum solutions with 0% added sodium chloride were stable. Generally, no intact whey - 0.15% xanthan gum solutions were stable either. Results observed for intact whey protein - 0.25% xanthan gum solutions at pH 7 were similar to those reported by Hidalgo and Hansen (1969). Hidalgo and Hansen (1969) found that solutions of β - lactoglobulin- 0.20% CMC at low ionic strength precipitated . They observed that 93% of the CMC was bound to the protein in the precipitate. Hidalgo and Hansen (1969) also noted that the addition of excess CMC resulted in a soluble complex being formed between the protein and the polysaccharide. Table 9 indicates that increasing the xanthan gum concentration to 0.25% resulted in stable solutions. Based on Hidago and Hansen's (1969) findings, it is reasonable to assume that the intact whey protein at pH 7 formed soluble complexes with the 0.25% xanthan gum. Increasing the xanthan gum concentration to 0.25% increased the net negative charge on the solution environment. It can be hypothesized that the increased net negative charge was responsible for charge repulsion, which prevented the intact whey protein - xanthan gum complexes from aggregating.

Table 9

## PERCENTAGE OF STABLE WHEY PROTEIN - XANTHAN GUM SOLUTIONS

INTACT WHEY pH 7

| GUM (%) | n | SODIUM CHLORIDE CONCENTRATION (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| 0.05 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.15 | 6 | 0 | 0 | 0 | 33 | 0 | 0 |
| 0.25 | 6 | 0 | 100 | 66 | 100 | 100 | 66 |

n= six repetitions per variable

Table 10

## PERCENTAGE OF STABLE WHEY SOLUTIONS

INTACT WHEY pH 5

| GUM (%) | n | SODIUM CHLORIDE CONCENTRATION (%) | | | | | |
|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | 4 | 5 |
| 0.05 | 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 0.15 | 6 | 0 | 100 | 100 | 66 | 33 | 0 |
| 0.25 | 6 | 0 | 0 | 100 | 66 | 100 | 100 |

n = six repetitions per variable

Hidalgo and Hansen (1969) observed that in the presence of CMC, β - lactoglobulin precipitates. They noted further that increasing the ionic strength to 0.20 molar essentially eliminated any precipitation of - lactoglobulin. As seen in Table 9, whey protein -0.25% xanthan gum solutions with only 1% added sodium chloride were stable. These solutions were 0.17 molar and thus were approaching the 0.20 molarity that Hidalgo and Hansen (1969) found would stabilize solutions of β - lactoglobulin and CMC. According to results of Hidalgo and Hansen (1969), it is expected that at the higher sodium chloride concentrations, the intact whey protein - 0.25% xanthan gum solutions would be stable. It should be noted that the intact whey protein - 0.25% xanthan gum solutions were stable when as much as 5% sodium chloride was added. It is hypothesized that the intact whey protein, at pH 7, hydrogen bonded to the xanthan gum. The helical structure of xanthan gum, basically unaffected by changes in salt concentration or pH, protected the intact whey protein from being salted - out by the higher levels of sodium chloride.

Table 10 shows the stability of intact whey protein - xanthan gum solutions at pH 5. Whey protein - 0.15% xanthan gum solutions at pH 5 were stable while those at pH 7 were not. At pH 5, whey protein is approaching its isoelectric point. Therefore it would be expected to form aggregates that precipitate. However, hydrogen bonding of the xanthan

gum to the intact whey protein appears to inhibit precipitation. The stable helical structure of xanthan gum stabilized the whey protein - 0.15% xanthan gum complexes. As the sodium chloride concentration increased beyond 2%, the stability of the whey protein - 0.15% xanthan gum solutions decreased. It is proposed that not enough gum was present in the system to protect whey from being salted - out by the increased concentrations of sodium chloride.

Table 10 indicates that increasing xanthan gum to 0.25% resulted in improved stability when compared to similar whey protein, pH 5 solutions with 0.15% xanthan gum. It is hypothesized that the whey protein attached itself to the xanthan gum structure via hydrogen bonding. At 0.25%, the xanthan gum was present in sufficient quantity to bind the majority of the whey protein and thereby protect it from being salted out at the higher sodium chloride concentrations.

It is interesting to compare Tables 5 and 9 for differences between the reaction of intact soy and intact whey protein to xanthan gum. At 3 to 5% added sodium chloride, the soy protein - 0.15% xanthan gum solutions formed soluble complexes. Whey protein - 0.15% xanthan gum solutions precipitated. The difference is explained by the effects of salt on protein. Soy protein is salted - in by higher ionic strengths. Whey protein is salted - out by higher ionic strengths (Shen,1981). Therefore, increasing to

around 3% sodium chloride unfolded the soy protein for interaction with xanthan gum; however, at the lower concentrations of xanthan gum, 3% sodium chloride caused the whey protein to aggregate and thereby prevented soluble whey protein - xanthan gum complexes from forming. When added to 0.25% xanthan gum, the whey protein solutions required less sodium chloride to stabilize than did the intact soy protein solutions. Whey protein - 0.25% xanthan gum solutions required only 1% added sodium chloride for stability; soy protein - 0.25% xanthan gum solutions required 3% added sodium chloride. Again, this difference is explained by the fact that soy protein is salted - in, while whey protein is salted - out by higher concentrations of sodium chloride.

Comparing Tables 7 and 10 shows the differences between intact soy protein - xanthan gum interactions and intact whey protein - xanthan gum interactions at pH 5. The major difference was that intact whey protein solutions were stable when blended with 0.15% xanthan gum while intact soy protein solutions were not stable with 0.15% xanthan gum. Whey proteins are smaller than soy proteins. It is perhaps the fact that the whey proteins were smaller than the soy that allowed the whey to fit better into the xanthan gum structure and thus be stabilized. If the protein can be bound to the gum, the helical structure of xanthan gum can protect the protein from precipitating. There was little difference between whey protein and intact soy protein solutions when

blended to the 0.25% level of xanthan gum. The only difference was that the soy solutions required only 1% added sodium chloride for stability; whereas the solutions with whey protein required 2% added sodium chloride. pH 5 approaches the isoelectric point of both whey and soy protein. pH 5 is somewhat closer to the isoelectric point of whey, 4.5, than to that of soy protein, 4.2. As pH 5 is approaching the isoelectric point, there is relatively little net charge on either the whey or the soy proteins. Therefore, both types of protein were compact and inclined to precipitate. The charged residues of the xanthan gum in combination with the added sodium chloride altered the ionic environment such that both the soy and whey proteins unfolded and thus formed soluble complexes with the xanthan gum. The fact that pH 5 was closer to the isoelectric point of whey protein than to that of soy, could explain why slightly more sodium chloride was needed to stabilize the whey protein - 0.25% xanthan gum solutions than to stabilize the soy protein - 0.25% xanthan gum solutions.

3. Effects of pH Variation

Differences were observed between the stability of soy protein - xanthan gum solutions at pH 7 and those solutions at pH 5. To further study the effects of pH on stability, intact and hydrolyzed soy protein - 0.25% xanthan gum solutions with 3% added sodium chloride were brought to pH's

2, 3, 4, 5, 7, and 9. The differences observed in solution stability are summarized in Table 11.

From Table 11 it can be seen that for solutions of intact soy, stability was lost as the pH extremes were approached. The excessive repulsive forces at the pH extremes may have caused the intact soy protein to be relatively inflexible. Ledward (1979) emphasizes the importance of flexibility in protein - polysaccharide interactions. The more flexible the protein, the better able the protein is to configure itself for maximum interaction with the gum. It is hypothesized that at pH extremes, the soy protein was either too inflexilble to complex with xanthan gum or the soy protein was precipitated by the acidic or basic environment.

Table 11 also provides the stability results of hydrolyzed soy protein - 0.25% xanthan gum solutions with 3% sodium chloride at various pH's. In general, the solutions with hydrolyzed soy were not as stable as those of intact soy. The hydrolyzed soy proteins are smaller than the larger intact soy proteins. It is hypothesized that the hydrolyzed soy proteins were capable of maximum hydrogen bonding to the xanthan gum. When the hydrogen bonding between the hydrolyzed soy and xanthan gum was at its maximum, large aggregates resulted. The soy protein - xanthan gum aggregates, because of their large molecular weight, resulted in insoluble soy protein - xanthan gum complexes.

Table 11

PERCENTAGE OF STABLE SOLUTIONS
WITH 0.25% XANTHAN GUM
AND 3% SODIUM CHLORIDE

| pH | n | SOY | |
|---|---|---|---|
| | | INTACT | HYDROLYZED |
| 2 | 6 | 33 | 67 |
| 3 | 6 | 67 | 33 |
| 4 | 6 | 83 | 67 |
| 5 | 6 | 83 | 67 |
| 7 | 6 | 100 | 67 |
| 9 | 6 | 33 | 33 |

n = six repetitions per variable

## C. RHEOLOGICAL PARAMETERS OF SOY PROTEIN-XANTHAN GUM SOLUTIONS

Rheological parameters were determined only for those solutions that remained stable. The only solutions that were stable at 0.05% xanthan gum were those of hydrolyzed soy at pH 5. For this reason, there is little data pertaining to solutions with 0.05% xanthan gum. The rheological parameters of these and other solutions are discussed below by pH.

### 1. Solutions at pH 7

The results of solutions at pH 7 are summarized in Tables 12 and 13. In these tables, flow behavior, n, is an index of pseudoplasticity. It can be seen that both the intact and hydrolyzed soy protein - xanthan gum solutions were similar in pseudoplasticity to their corresponding controls. The 0.15% xanthan gum control had a flow behavior index of 0.42. Intact and hydrolyzed soy protein - 0.15% xanthan gum solutions all had flow behavior indices ranging between 0.44 and 0.46, slightly less pseudoplastic.

It is the helical structure and super junction zones of xanthan gum that make it pseudoplastic (Kelco, 1975). It is hypothesized that soy protein complexed with xanthan gum and fit into the gum's helical structure. According to Dea (1977), galactomannans complex with xanthan gum in such a manner and cause a

Table 12

MEAN FLOW BEHAVIOR INDICES AND CONSISTENCY COEFFICIENTS OF INTACT SOY PROTEIN - XANTHAN GUM SOLUTIONS AT pH 7

| SODIUM CHLORIDE(%) | FLOW BEHAVIOR | | CONSISTENCY | |
|---|---|---|---|---|
| | 0.15% XG | 0.25% XG | 0.15% XG | 0.25% XG |
| CONTROL | 0.42 | 0.34 | 190.0 | 685.0 |
| 0 | * | * | * | * |
| 1 | 0.45 | 0.31 | 261.1 | 777.9 |
| 2 | 0.45 | 0.33 | 267.9 | 941.2 |
| 3 | 0.44 | 0.31 | 271.1 | 917.8 |
| 4 | 0.46 | 0.32 | 311.7 | 942.0 |
| 5 | 0.45 | 0.34 | 381.4 | 955.8 |

* No stable solutions

Table · 13

MEAN FLOW BEHAVIOR INDICES AND CONSISTENCY COEFFICIENTS OF HYDROLYZED SOY PROTEIN-XANTHAN GUM SOLUTIONS AT pH 7

| SODIUM CHLORIDE(%) | FLOW BEHAVIOR | | CONSISTENCY | |
|---|---|---|---|---|
| | 0.15% XG | 0.25% XG | 0.15% XG | 0.25% XG |
| CONTROL | 0.42 | 0.34 | 190.0 | 685.0 |
| 0 | * | * | * | * |
| 1 | 0.45 | 0.35 | 249.1 | 742.8 |
| 2 | 0.45 | 0.35 | 240.0 | 780.8 |
| 3 | 0.44 | 0.33 | 291.9 | 868.8 |
| 4 | 0.44 | 0.33 | 268.1 | 861.8 |
| 5 | 0.44 | 0.31 | 296.4 | 950.8 |

* No stable solutions

synergistic reaction. Dea (1977) does not explain specifically what caused the viscosity synergism between xanthan gum and galactomannans. It is proposed that soy protein could react with xanthan gum in a fashion similar to that of galactomannans. In doing so, the soy would fit into the gum structure without affecting the super juction zones. The 0.25% xanthan gum control had a flow behavior index of 0.34. The flow behavior of soy protein - 0.25 xanthan gum solutions had flow behavior indices ranging from 0.31 to 0.35.

It is proposed that in stable soy protein - xanthan gum solutions the soy binds to xanthan gum in a manner similar to the way galactomannans bind to xanthan gum (Dea, 1977). Dea (1977) proposed that galactomannans become entangled in the xanthan gum structure thus causing a viscosity synergism. It is proposed that soy protein had the same synergistic interaction with xanthan gum. The viscosity synerisms observed, indicated in Tables 12 and 13 as increased consistency coefficients, support this proposal. When complexed with xanthan gum, soy protein prevented the close alignment of xanthan gum helices. By preventing this alignment and by contributing to the hydrodynamic volume of the gum molecule, soy protein reacted synergistically with xanthan gum.

Tables 12 and 13 it can be seen that the consistency coefficients of the intact and hydrolyzed soy protein - 0.15% xanthan gum solutions were greater than that of the 0.15% xanthan gum control. The consistency coefficient of the 0.15% xanthan gum control was 190.0 cps. The consistency coefficients of the intact soy protein - 0.15% xanthan gum solutions ranged from 261 cps to 381.4 cps, while solutions of hydrolyzed soy - 0.15 % xanthan gum ranged from 240.0 to 296.4 cps. Solutions of intact soy - 0.25% xanthan gum ranged in apparent viscosity from 777.9 to 955.88 cps. Solutions of hydrolyzed soy - 0.25% xanthan gum ranged in apparent viscosity from 742.8 to 950.8 cps. As seen in Figure 15, all the soy protein-0.25 % xanthan gum solutions were more viscous than the control, which had a consistency coefficient of 685.0 cps. Figure 15 also illustrates that there was little difference between solutions with intact soy and hydrolyzed soy. It can be seen that the consistency coefficient of solutions made with intact soy were generally 20 to 100 cps greater than those of solutions made with hydrolyzed soy. Molecules of intact soy are larger than those of hydrolyzed soy. Therefore, once bound to the xanthan gum the larger soy proteins did more to disrupt the helical structure of xanthan gum than did molecules of hydrolyzed soy. Due to their smaller size, molecules of

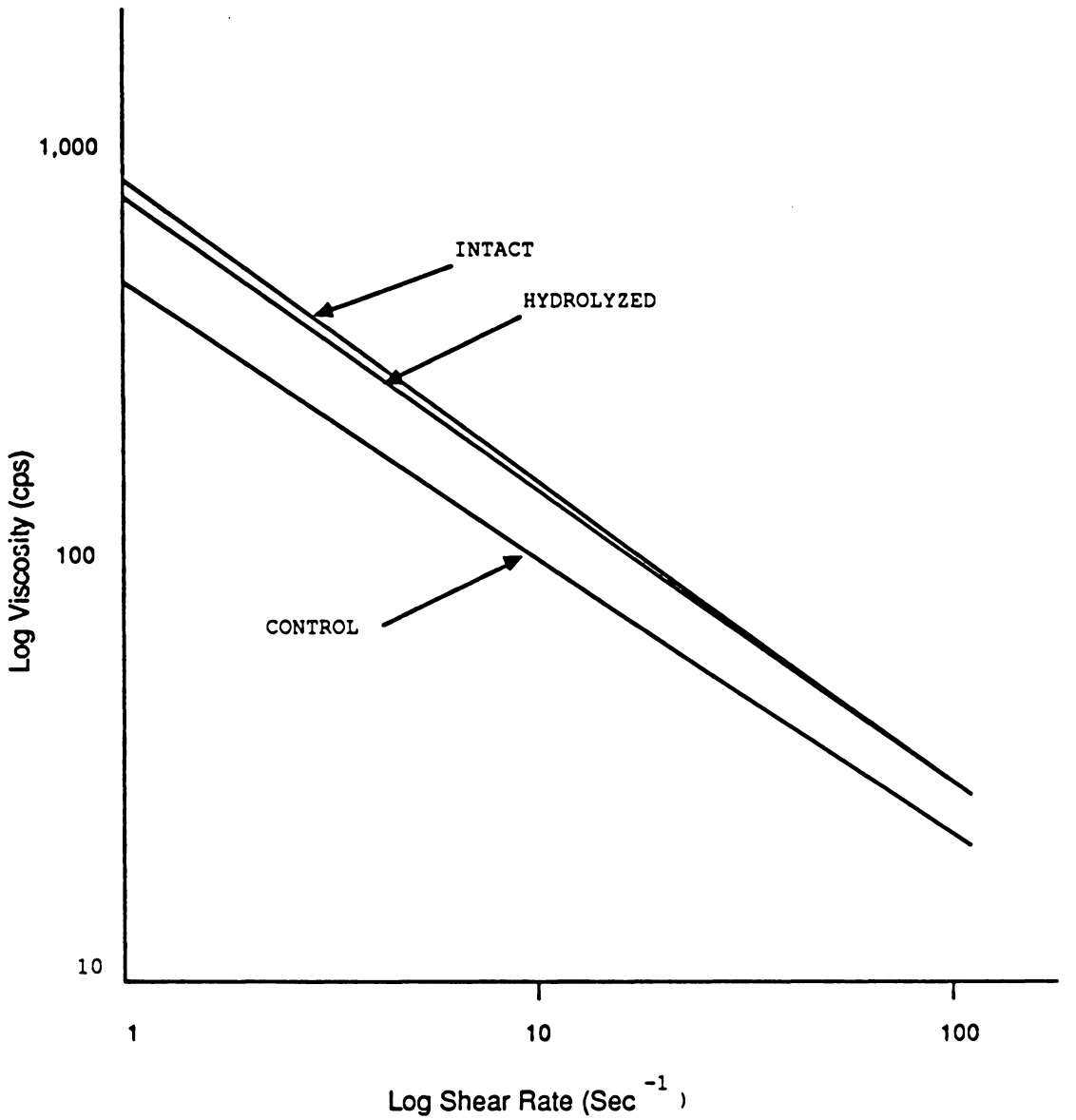


**Figure 15. Apparent Viscosity of Intact versus Hydrolyzed Soy Protein - 0.25 % Xanthan Gum Solutions with 3% Added Sodium Chloride at pH 7**

hydrolyzed soy did little to disrupt the structure of the gum; therefore, hydrolyzed soy did not increase the apparent viscosity as much as did the unhydrolyzed soy. As the viscosities of all the 2% soy protein solutions alone were little more than 3 cps, the high viscosities observed are attributable to a synergism between the soy protein and xanthan gum.

From tables 12 and 13 indicate that the consistency coefficient of the intact soy protein - 0.15 and 0.25% xanthan gum solutions increased as the sodium chloride concentration increased from 1 to 5%. The higher levels of sodium chloride result in a salting - in of soy protein. Sodium chloride levels above 0.1 and 0.2 molar for intact and hydrolyzed soy protein, respectively, cause soy protein to unfold (Shen, 1981). When unfolded, more soy protein segments were available for interaction with xanthan gum. The more soy that complexed with the gum, the greater the observed viscosity synergism. The viscosity synergism could be the result of soy protein increasing the dynamic volume of the gum molecule.

2. Solutions at pH 5

To further study the interaction between soy protein and xanthan gum at pH 5, soy protein - xanthan gum solutions at pH 5, with 0 to 5% added sodium

chloride were prepared. Tables 14 and 15 summarize the rheological parameters observed for soy protein - xanthan gum solutions at pH 5 with 0 to 5% added sodium chloride.

The results seen in Table 14 refer to solutions made with intact soy. No intact soy protein - 0.05% xanthan gum solutions at pH 5 were stable; therefore no rheological data appear for these solutions. It is apparent from Table 14 that the flow behavior, n, was essentially unaffected by the addition of soy protein and sodium chloride to xanthan gum. This consistency in flow behavior indices was also observed at pH 7.

Table 14 indicates large increases in consistency coefficients, m, when intact soy protein and sodium chloride were added to xanthan gum. It is proposed that these increases resulted when soy protein bound to the helical structure of xanthan gum. In so binding to the gum, the protein increased the dynamic volume of the molecule as a whole, and hence caused an increased apparent viscosity. It is proposed that the soy protein joined with the xanthan gum via hydrogen bonding.

It should be noted in Table 14 that increased sodium chloride resulted in increased apparent viscosity. This increase is illustrated in Figure 16. It is proposed that the increased viscosity observed with added sodium chloride at pH 5 resulted for the same

Table 14

MEAN FLOW BEHAVIOR INDICES AND CONSISTENCY COEFFICIENTS OF INTACT SOY PROTEIN - XANTHAN GUM SOLUTIONS AT pH 5

| SODIUM CHLORIDE(%) | FLOW BEHAVIOR | | CONSISTENCY | |
|---|---|---|---|---|
| | 0.15% XG | 0.25% XG | 0.15% XG | 0.25% XG |
| CONTROL | 0.42 | 0.34 | 190.0 | 685.0 |
| 0 | 0.51 | 0.34 | 169.2 | 695.8 |
| 1 | 0.44 | 0.32 | 277.0 | 1088.7 |
| 2 | 0.45 | 0.33 | 324.5 | 1092.6 |
| 3 | 0.46 | 0.34 | 298.4 | 1110.3 |
| 4 | 0.46 | 0.33 | 343.6 | 1183.7 |
| 5 | 0.41 | 0.34 | 396.7 | 1136.4 |

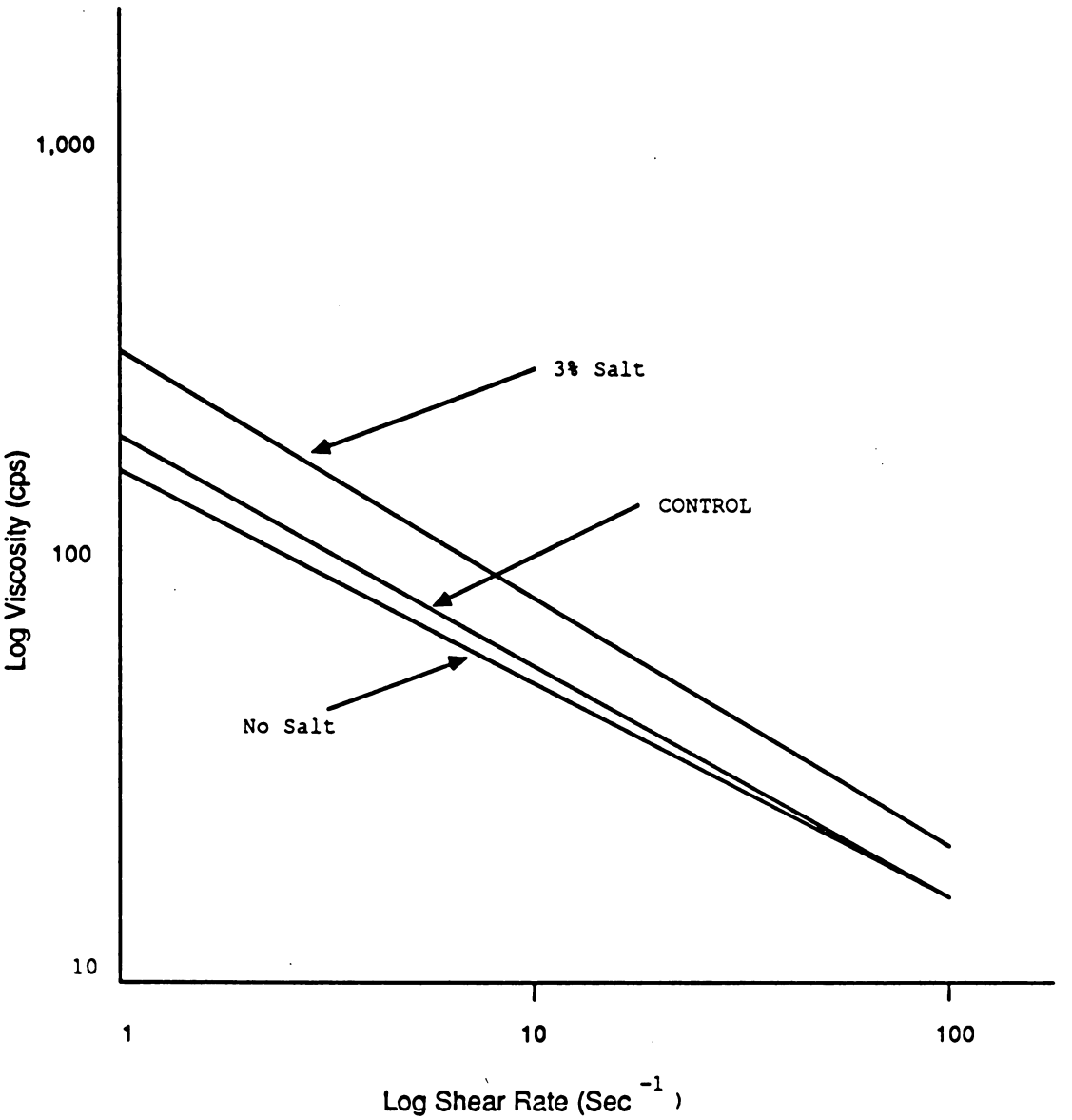


Figure 16. Apparent Viscosity of Intact Soy Protein - 0.15 % Xanthan Gum Solutions at pH 5 with and without Added Sodium Chloride

reasons that it did at pH 7. Sodium chloride levels above 0.1 and 0.2 molar for intact and hydrolyzed soy protein, respectively, cause soy protein to unfold (Shen, 1981). When unfolded, more soy protein segments were available for interaction with xanthan gum. The more soy that complexed with the gum, the greater the observed viscosity synergism. The viscosity synergism would be the result of soy protein increasing the dynamic volume of the gum molecule.

A comparison of Tables 12 and 14 indicates that intact soy protein - xanthan gum solutions at pH 5 were generally higher in apparent viscosity than similar solutions at pH 7.

It is interesting that as the soy protein approached its isoelectric range, the viscosity synergism with xanthan gum was at its maximum. Similar finding were reported by Ganz (1974) in his study of gelatin - CMC solutions. At pH 5 soy protein tends to aggregate. It is proposed that these aggregates were soluble. Furthermore, it is proposed that the soy protein aggregates served to increase the dynamic volume of the soy protein - xanthan gum complexes more so than soy protein at pH 7.

Figure 17 compares rheograms for solutions of intact soy protein - 0.15% xanthan gum solutions with 3% added sodium chloride at pH's 7 and 5. It can be seen

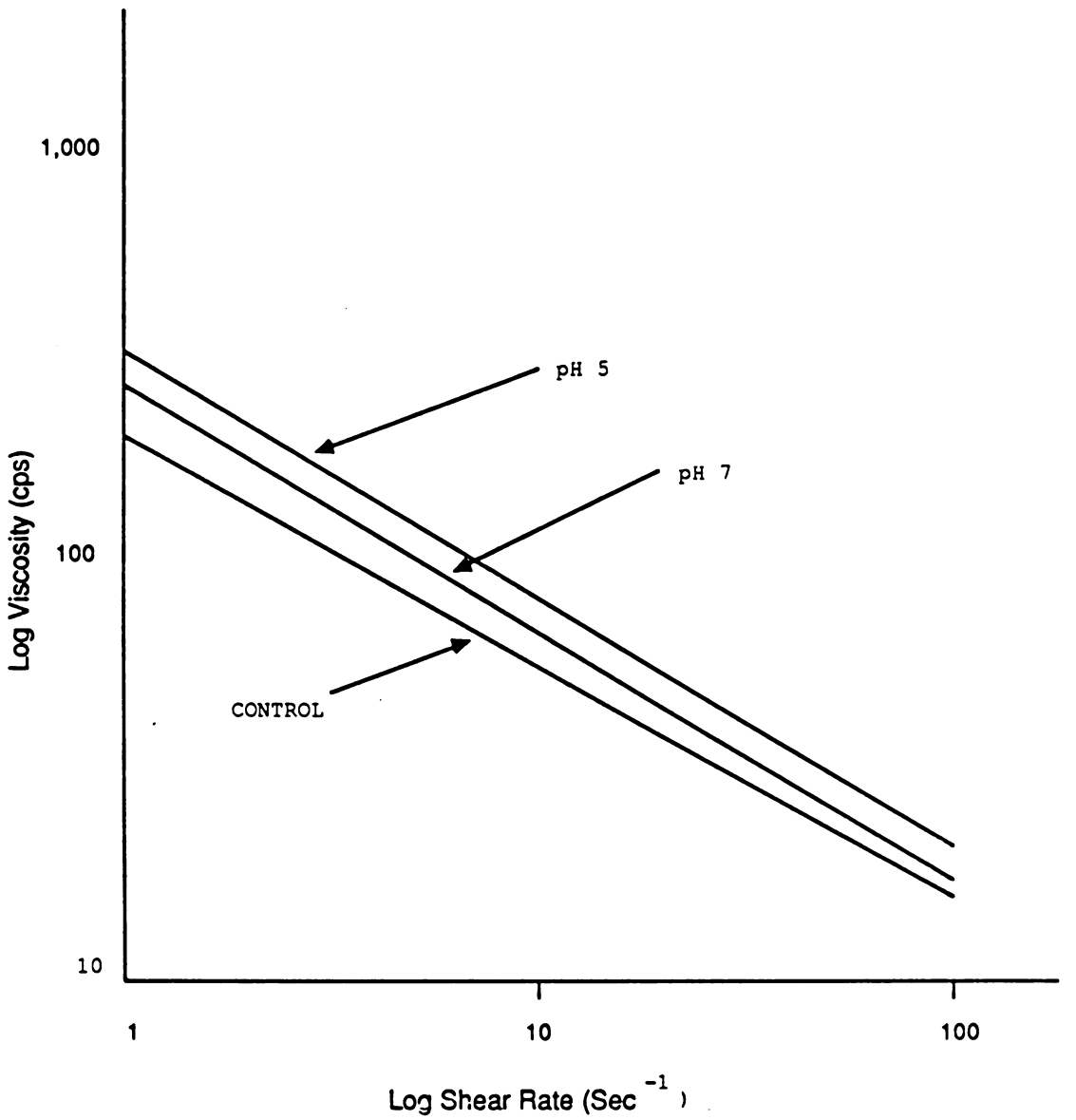


**Figure 17. Apparent Viscosity of Intact Soy Protein - 0.15 % Xanthan Gum Solutions with 3 % Added Sodium Chloride at pH 5 and 7**

that the solution at pH 5 was more viscous than either the control or the solution at pH 7. When Tables 12 and 14 are compared it is seen that intact soy protein - 0.25% xanthan gum solutions at pH 5 were also higher in apparent viscosity than similar solutions at pH 7. Figure 18 illustrates rheograms for the intact soy 0.25% xanthan gum solutions with 1% added sodium chloride at pH's 7 and 5.

Viewing Table 15, it is interesting to note that hydrolyzed soy protein - 0.05% xanthan gum solutions at pH 5 were the only solutions stable at this low gum concentration. Table 15 indicates that the flow behavior index, n, of the control was 0.44. It also indicates that the soy caused the flow behavior to increase to between 0.69 and 0.76. These were the only stable solutions studied that evidenced a flow behavior significantly greater than the control. It is proposed that at this lower concentration of xanthan gum, the soy protein interfered with the gum's super junction zones. It is suggested that the small molecules of hydrolyzed soy hindered the allignment of the super junction zones and thereby reduced the pseudoplasticity. It should be recalled that an increase in the flow behavior index, n, relates to a decrease in pseudoplasticity.

When the xanthan gum concentration was raised to

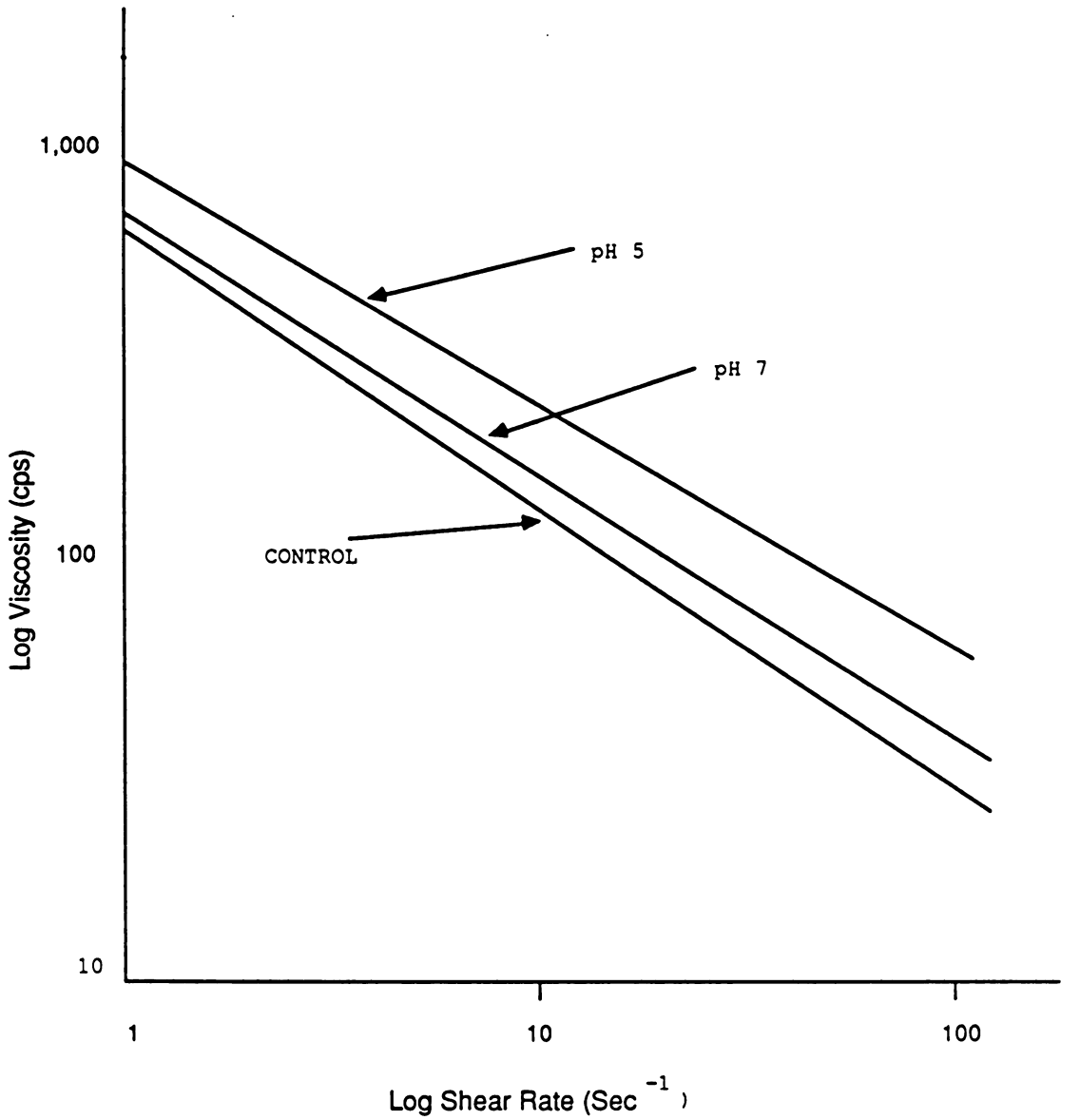


**Figure 18. Apparent Viscosity of Intact Soy Protein - 0.25 % Xanthan Gum Solutions with 3 % Added Sodium Chloride at pH 5 and 7**

Table 15

Mean Flow Behavior Indices and Consistency Coefficients of Hydrolyzed Soy Protein-Xanthan Gum Solutions at pH 5

| SODIUM CHLORIDE(%) | FLOW BEHAVIOR | | | CONSISTENCY | | |
|---|---|---|---|---|---|---|
| | 0.05% XG | 0.15% XG | 0.25% XG | 0.05% XG | 0.15% XG | 0.25% XG |
| CONTROL | 0.44 | 0.42 | 0.34 | 12.3 | 190.0 | 685.0 |
| 0 | 0.76 | 0.44 | 0.34 | 22.3 | 274.0 | 864.7 |
| 1 | 0.74 | 0.43 | 0.34 | 23.9 | 285.5 | 912.3 |
| 2 | 0.75 | 0.43 | 0.34 | 24.2 | 295.9 | 915.9 |
| 3 | 0.74 | 0.44 | 0.34 | 25.6 | 314.3 | 925.3 |
| 4 | 0.69 | 0.43 | 0.33 | 31.5 | 294.0 | 973.1 |
| 5 | * | 0.43 | 0.34 | * | 319.5 | 960.9 |

* No stable solutions

0.15 and 0.25 %, neither intact nor hydrolyzed soy protein effected the flow behavior index. It is proposed that at 0.15 and 0.25 % gum, not enough soy protein was present to hinder the alignment of the super junction zones.

Based on the results observed for solutions of hydrolyzed soy at pH 7, it is not surprizing to see that all the hydrolyzed soy protein - xanthan gum solutions were greater in apparent viscosity than the appropriate control. A comparison of Tables 14 and 15 indicated that pH 5 solutions made with hydrolyzed soy were lower in apparent viscosity than similar solutions made from intact soy protein at pH 5. This is illustrated in Figure 19. Comparing Figures 15 and 19, it can be seen that there is more of a difference in viscosity between intact and hydrolyzed soy at pH 5 than at pH 7.

3. Solutions at pH 2,3,4,5,7,and 9

Table 16 indicates that pH effected the rheological parameters of intact and hydrolyzed soy protein - 0.25% xanthan gum solutions with 3% added sodium chloride. The flow behavior index was affected by pH. At pH's 2 and 3 the flow behavior, n, of the intact soy solutions was 0.46 and 0.41, differing from the control by 0.12 and 0.07, respectively. At pH's 2 and 3 the flow behavior of the hydrolyzed soy solutions was even

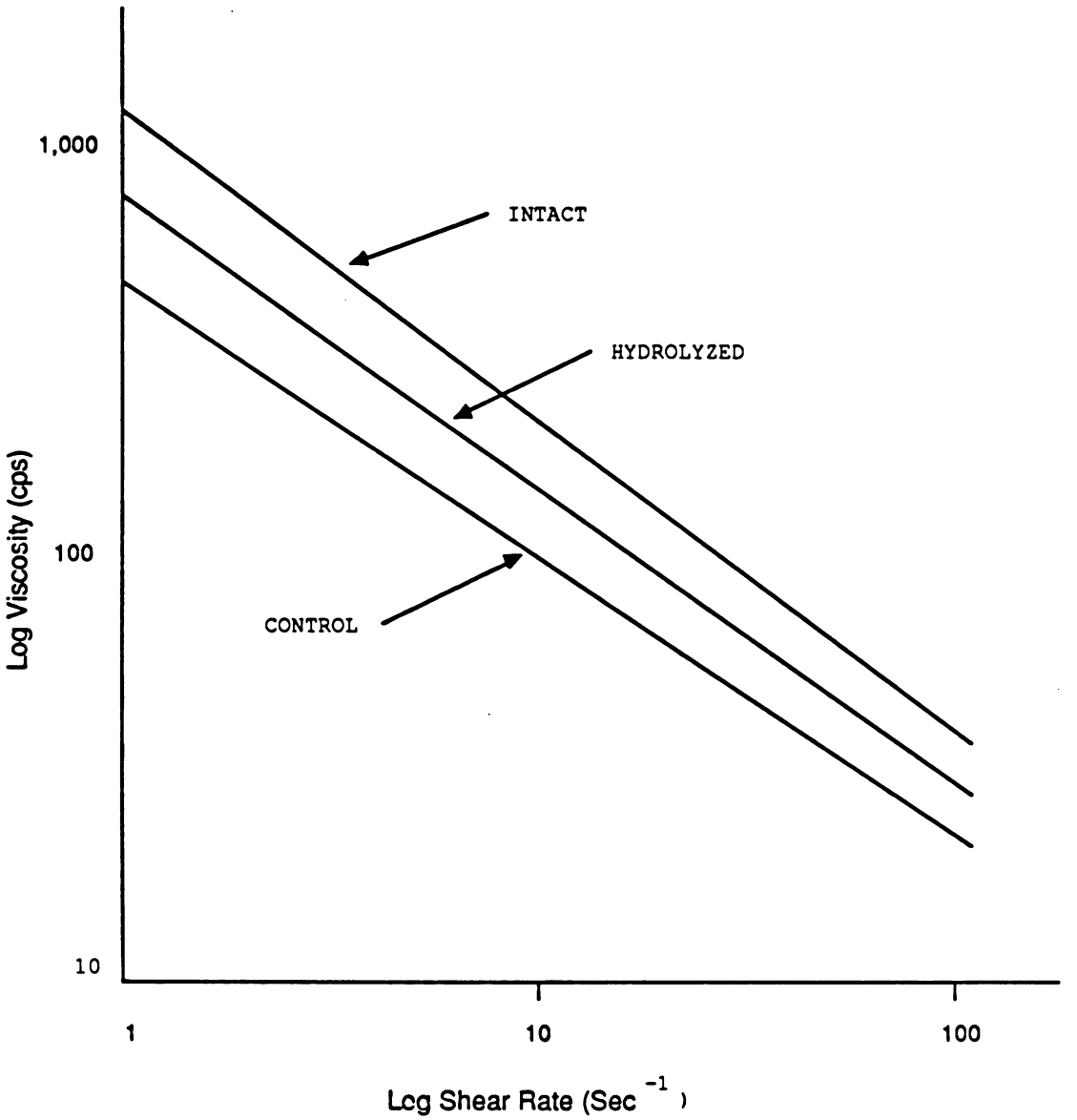


**Figure 19. Apparent Viscosity of Intact versus Hydrolyzed Soy Protein - 0.25 % Xanthan Gum Solutions with 3% Added Sodium Chloride at pH 5**

Table 16

MEAN FLOW BEHAVIOR INDICES AND CONSISTENCY COEFFICIENTS OF INTACT AND HYDROLYZED SOY PROTEIN - 0.25% XANTHAN GUM. SOLUTIONS AT VARIOUS pH'S *

| pH | FLOW BEHAVIOR | | CONSISTENCY | |
|---|---|---|---|---|
| | Intact | Hydrolyzed | Intact | Hydrolyzed |
| CONTROL | 0.34 | 0.34 | 685.0 | 685.0 |
| 2 | 0.46 | 0.53 | 497.3 | 235.3 |
| 3 | 0.41 | 0.44 | 482.1 | 350.3 |
| 4 | 0.31 | 0.36 | 1262.1 | 690.6 |
| 5 | 0.34 | 0.34 | 1110.3 | 925.3 |
| 7 | 0.31 | 0.33 | 271.1 | 868.8 |
| 9 | 0.33 | 0.35 | 884.9 | 698.3 |

* Sodium chloride levels at 3% for all solutions

higher. They were 0.53 and 0.44 at pH's 2 and 3, respectively. At these acidic pH extremes the environment was highly protonated. It is proposed that charge repulsion hindered the ability of the super junction zones to reallign. In so disrupting the gum's super junction zones, pseudoplasticity, as indicated by flow behavior, was reduced. Table 16 indicates that flow behavior was essentially unaffected by pH's from 4 to 9. Table 16 reports considerable variation in the consistency coefficients. The consistency coefficient, m, of the 0.25% xanthan gum control was 685.0 cps. As seen in Table 16, some of the intact soy - 0.25% xanthan gum solutions had consistency coefficients less than that of the control. At pH 2 and 3, the consistency coefficients were 497.3 and 482.1 cps, respectively. As evidenced by their low consistency coefficients, these solutions were both significantly less viscous than the control.

According to Kelco (1975), the apparent viscosity of xanthan gum is unaffected by pH. However, results displayed in Table 16 indicate that soy protein - 0.25% xanthan gum solutions are affected by pH. At the acidic extremes of pH 2 and 3, the solution environments were highly protonated. The carboxylate groups of the xanthan gum may have combined with the hydrogen ions in the solution. This would leave the gum unavailable for

interaction with the soy protein. The decrease in viscosity suggests that the soy protein and xanthan gum did occasionally interact to form insoluble complexes. This would have had the effect of reducing the concentration of soluble xanthan gum and therefore of reducing the apparent viscosity. Results of the current research agree with finding of Ganz (1974). Ganz (1974) reported that at the isoelectric point for gelatin, the gelatin and carboxymethylcellulose, CMC, complexed and increased in viscosity. Decreasing the pH below the isoelectric range of gelatin resulted first in a viscosity decrease, then in precipitation.

Table 16 indicated a maximum consistency coefficient, m, at pH 4 and 5 for solutions of intact soy protein - 0.25% xanthan gum solutions with 3% added sodium chloride. This agreed with the finding by Ganz (1974) that the maximum viscosity of a gelatin - CMC solution occurred in the isoelectric range of the gelatin. Intact soy protein - 0.25% xanthan gum solutions at pH 4, had a consistency coefficient of 1262.1 cps, which was 577.1 cps greater than the control. At pH 5, m was 1110.3 cps, which was 425.3 cps greater than the control. It is interesting that maximum viscosity occurred in the isoelectric range of soy protein. At its isoelectric point, inter- and

intramolecular binding result in aggregates of soy. It is proposed that the large soy aggregates increased the dynamic volume of the soy protein - xanthan gum complexes, and therefore, increased the viscosity of the solution.

At pH 7 and 9, soy protein was more unfolded than it was in its isoelectric range. It is hypothesized that because it was somewhat unfolded, the soy protein molecules conformed more to the gum's structure, alligning with the helices and forming fewer branches than were formed at pH's 4 and 5. The formation of fewer branches resulted in less of an increase in dynamic volume and in less viscosity synergism.

From Table 16 it can also be seen that solutions of hydrolyzed soy protein - 0.25% xanthan gum with 3% added sodium chloride were affected by pH. pH's 2 and 3 produced solutions with the lcwest consistency coefficients and, therefore, the lowest apparent viscosities. The fact that the consistency coefficients were 235.3 and 350.3 cps at pH's 2 and 3, respectively should be noted. These were 449.7 and 334.7 cps less than the 0.25% xanthan gum control, which had a consistency coefficient of 685.0 cps.

When blended with 0.25% xanthan gum at various pH's, solutions of hydrolyzed soy protein were less viscous than similar solutions with intact soy protein.

Molecules of hydrolyzed soy protein are smaller than molecules of intact soy. It is proposed that the smaller molecules of hydrolyzed soy did not increase the hydrodynamic volume of the soy protein - xanthan gum complex as much as did molecules of intact soy.

D. PERCENT SOLUBLE PROTEIN MEASUREMENTS

The objective of this portion of the study was to determine whether the amount of soluble protein in solution correlated with stability. It was hypothesized that solutions that remained stable would be higher in soluble protein than those that precipitated. A review of the literature indicated that protein - polysaccharide complex formations and precipitation were not generally characterized; the general assumption was that precipitates were proteinaceous material. Hidalgo and Hansen (1969) did attempt to characterize the precipitate. They determined the extent to which beta - lactoglobulin was bound to CMC. The conclusions of Hidalgo and Hansen (1969) were that at maximum precipitation, the protein was nearly all bound to the gum. However, as precipitation decreased, so did the percentage of protein bound to gum.

The current study did not attempt to determine the amount of protein bound to gum. Instead, it attempted to determine percentages of soluble protein in given protein - polysaccharide solutions and the effects of pH, sodium chloride concentration, amd xanthan gum concentration on soluble protein. Finally possible correlations between soluble protein and solution stability were examined.

From Table 17, it can be seen that intact, pH 7, soy

Table 17 . Solubility Measurements of Intact and Hydrolyzed Soy Protein - Xanthan Gum Solutions at pH 7

| | | INTACT SOY pH 7 | | | HYDROLYZED SOY pH 7 | | |
|---|---|---|---|---|---|---|---|
| | | XANTHAN GUM (%) ± S.D. | | | XANTHAN GUM (%) ± S.D. | | |
| NaCl% | n | 0.05 | 0.15 | 0.25 | 0.05 | 0.15 | 0.25 |
| 0 | 6 | 40.8 ± 2.5 | 44.4 ± 6.0 | 51.8 ± 1.4 | 77.9 ± 7.3 | 69.4 ± 2.3 | 97.4 ± 1.1 |
| 1 | 6 | 30.5 ± 4.2 | 46.7 ± 5.5 | 68.3 ± 4.0 | 71.8 ± 2.3 | 70.1 ± 2.3 | 94.9 ± 4.4 |
| 2 | 6 | 30.2 ± 2.7 | 47.3 ± 2.8 | 83.0 ± 5.8 | 72.7 ± 1.6 | 68.8 ± 2.0 | 96.3 ± 2.5 |
| 3 | 6 | 39.8 ± 6.7 | 48.9 ± 3.4 | 131.5 ± 7.4 | 68.7 ± 3.4 | 73.7 ± 4.4 | 100.7 ± 3.9 |
| 4 | 6 | 33.0 ± 3.9 | 48.5 ± 1.7 | 101.1 ± 6.0 | 69.9 ± 1.1 | 70.3 ± 2.6 | 94.8 ± 8.2 |
| 5 | 6 | 31.0 ± 5.4 | 51.5 ± 3.0 | 98.9 ± 20.5 | 71.7 ± 3.2 | 73.5 ± 1.5 | 90.8 ± 11.2 |

with 0.05% xanthan gum had less than 40% soluble protein. Therefore, over 60% of the intact, pH 7 soy was in the precipitate. Increasing gum concentration to 0.15%, resulted in about 50% soluble protein. It was not until a level of 0.25% xanthan gum that soluble protein increased above 50%. Table 17 indicates that sodium chloride appeared to affect the solubility of intact soy protein - 0.25% xanthan gum solutions. At 0 % added sodium chloride there was 51.8% soluble protein. As sodium chloride was increased to 1, 2, and 3%, soluble protein increased to 68, 83, and 131.5% respectively. It is known that higher concentrations of sodium chloride cause soy protein to be more soluble (Shen, 1980). Therefore, the observed results are what were expected. Increasing from 3 to 4 and 5% sodium chloride resulted in reducing the soluble protein from 131.5 to 101.1 and 98.9 %, respectively. It is hypothesized that the excess sodium chloride altered the ionic environment such that insoluble soy protein - xanthan gum complexes were formed. Table 17 also illustrates that within any given sodium chloride level, soluble protein increased with increased concentration of xanthan gum. This increase is attributable to the charged residues on the xanthan gum and their effect on the overall ionic environment of the solution. It should be noted that the increase in solubility due to the xanthan gum concentration was

greater as the sodium chloride concentration increased. At 1% added sodium chloride there was a difference of 37.8 % soluble protein as the xanthan gum was increased from 0.05 to 0.25%. At 3% added sodium chloride there was a difference of 91.7 % soluble protein as the gum was increased from 0.05 to 0.25 %. At 5% added sodium chloride, there was a slightly lower increase of 67.8 % soluble protein.

Table 18 compares results of visually determined stability and percent soluble protein. At 0.15 % xanthan gum, the percentage of soluble protein did not compare to visual stability. While the stability ranged from less than 50 % to 100 % , the percent soluble protein only ranged from 44 and 52 %. It is hypothesized that at 0.15 % xanthan gum, some of the soy protein formed soluble complexes with the xanthan gum. There are two possible explanations for the protein that was not soluble. The insoluble protein might have been bound with xanthan gum in the form of insoluble complexes. These insoluble complexes may have been suspended in the solution, thus showing no obvious precipitate. It is also possible that the insoluble protein was independent of the xanthan gum, but remained suspended due to the viscosity of the solution.

Table 18 also compares visually determined stability

**Table18. Comparison of Stability to Solubility Where at Least 50% of the Solutions are Stable**

| | INTACT SOY pH 7 | | | |
|---|---|---|---|---|
| | 0.15% Xanthan Gum | | 0.25% Xanthan Gum | |
| NaCl(%) | Stable(%) | Soluble(%) | Stable(%) | Soluble(%) |
| 0 | * | ** | * | ** |
| 1 | * | ** | * | ** |
| 2 | * | ** | 50 | 83 ± 6 |
| 3 | 50 | 49 ± 3 | 100 | 132 ± 7 |
| 4 | 100 | 49 ± 2 | 100 | 101 ± 6 |
| 5 | 83 | 52 ± 3 | 100 | 99 ± 20 |

* Less than 50% Stable

** Not applicable

to percent soluble protein for soy solutions with 0.25 % xanthan gum. At 0.25 % xanthan gum, soluble protein did relate to visual stability. It is hypothesized that the higher gum concentration of 0.25 % altered the ionic environment of the solution such that the soy protein and xanthan gum formed soluble complexes. Table 17 shows that as the sodium concentration increased from 0 % to 3 %, the percent soluble protein rose from 51.8 to 131.5 %. It is suggested that the increased charge contributed by the increased sodium chloride levels, enabled the formation of soluble soy protein - xanthan gum complexes. The 131.5 % soluble protein found in solutions with the 3 % added sodium chloride can be explained by the methodology used. When setting the standard, which was a 2% soy protein isolate solution with no xanthan gum, the insoluble protein fractions were precipitated by centrifugation. Therefore, the standard, against which the soy - xanthan gum solutions were compared, did not account for the insoluble soy protein fractions. In the soy protein - 0.25 % xanthan gum solutions with 3 % added sodium chloride, it is proposed that the ionic environment was such that all the soy protein fractions were stabilized by the gum. Thus, the percent soluble protein was greater than 100 % of the standard. From 3 % to 5% added sodium chloride, the percent soluble protein dropped somewhat, yet remained

close to 100%.

The interaction between xanthan gum and globular soy proteins may form weak, soluble complexes in a manner similar to the reaction between galactomannans and xanthan gum (Dea, 1974). It is proposed that through hydrogen bonding the soy protein and xanthan gum interacted. Centrifuging, which was a necessary part of the methodology for determining percent soluble protein, may have caused some hydrogen bonds to break, leaving the protein independent of the xanthan gum and allowing the protein once again to precipitate. Where the soy protein - xanthan gum interactions were strong, as in the soy protein - 0.25% xanthan gum with 3% added sodium chloride, centrifugation did not break the soy - xanthan gum bonds and, thus, high levels of soluble proteins were observed. In less stable solutions the centrifugation process may have caused weak soy protein - xanthan gum bonds to break. It is hypothesized that by breaking disulfide linkages between soy protein's 7S and 11S fractions, xanthan gum causes solubility to improve. Disulfide bonds may be reformed following the process of centrifugation.

Hermansson (1979) indicated that the complex quaternary structure of the 7S and 11S fractions lend these two protein fractions to association - dissociation

reactions. Xanthan gum itself is a helical structure that will also form aggregates. The two major components of the model systems involved in this study, soy protein and xanthan gum, were both dynamic in nature. Their ability to form structures, break, and reform structures indicates that whatever observations are made are only made at a given point in time. The dynamics of this system may be such that at two unique points in time the same system can be observed acting differently. Large standard deviations observed for mean percent solubility and indicated in Tables 17 through 22, suggest the dynamics of these systems is real.

From Table 19 it can again be seen that there is no relationship between percent soluble protein and solution stability. The correlation coefficient was -0.04. Solutions of hydrolyzed soy - 0.25% xanthan gum at pH 7 with no added sodium chloride had 97.4% soluble protein. However, these same solutions were not stable. It is suggested that the methodology for determining percent soluble protein may not have been appropriate for use with solutions of hydrolyzed soy protein. It should be recalled that the percentage soluble protein was based on a control sample of hydrolyzed soy protein that had most insoluble components separated out by centrifugation. Hydrolyzed soy protein - 0.25% xanthan gum solutions at pH 7 with 2% added sodium chloride had 96.3% soluble

## Table 19. Comparison of Stability to Solubility Where at Least 50 % of the Solutions are Stable

| | HYDROLYZED SOY pH 7 | | | | | |
|---|---|---|---|---|---|---|
| | 0.05 % Gum | | 0.15 % Gum | | 0.25 % Gum | |
| NaCl (%) | Stable (%) | Soluble (%) | Stable (%) | Soluble (%) | Stable (%) | Soluble (%) |
| 0 | * | ** | * | ** | * | ** |
| 1 | * | ** | * | ** | * | ** |
| 2 | * | ** | 67 | 69 + 2 | 50 | 96 + 3 |
| 3 | 67 | 69 ± 3 | 100 | 74 + 4 | 67 | 101 + 4 |
| 4 | * | ** | 100 | 70 + 3 | 67 | 95 + 8 |
| 5 | * | ** | 100 | 71 + 6 | 67 | 91 + 11 |

* Less than 50 % Stable

** Not Applicable

protein. However, as can be seen in Table 19 , these solutions were stable. A comparison of the hydrolyzed soy protein - 0.25% xanthan gum solutions with no added sodium and with 3% added sodium shows that there was no correlation between percent soluble protein and stability. It is proposed that in stable solutions the insoluble as well as soluble soy components may have bound to the xanthan gum and been incorporated into the gum's helical structure. With incorporation into the gum structure, the soy protein was prevented from precipitating. It appears that concentrations of at least 2% sodium chloride were necessary to enable the protein to bind to the xanthan gum. Sodium concentrations of 2% or more resulted in a salting - in of the hydrolyzed soy protein. As the hydrolyzed soy proteins were salted - in they unfolded. By their unfolding, the hydrolyzed soy proteins were more readily available for interaction with xanthan gum. When the soy protein was incorporated into the gum it could not participate in protein - protein interaction. Therefore protein aggregates did not form and precipitation did not result.

Tables 20, 21, and 22 summarize results of percent soluble protein for intact and hydrolyzed soy protein - xanthan gum solutions at pH 5. Table 20 indicates that the majority of intact soy protein - xanthan gum

Table 20 . Solubility Measurements of Intact and Hydrolyzed Soy Protein - Xanthan Gum Solutions at pH 5

| | | INTACT SOY pH 5 | | | HYDROLYZED SOY pH 5 | | |
|---|---|---|---|---|---|---|---|
| | | XANTHAN GUM (%) ± S.D. | | | XANTHAN GUM (%) ± S.D. | | |
| NaCl% | n | 0.05 | 0.15 | 0.25 | 0.05 | 0.15 | 0.25 |
| 0 | 6 | 44.8 ± 11.4 | 53.8 ± 20.5 | 55.7 ± 17.6 | 69.8 ± 2.4 | 67.5 ± 4.8 | 103.6 ± 5.8 |
| 1 | 6 | 36.7 ± 3.9 | 65.7 ± 11.6 | 82.4 ± 1.4 | 67.7 ± 4.9 | 74.0 ± 6.3 | 78.0 ± 15.4 |
| 2 | 6 | 48.4 ± 3.9 | 68.8 ± 8.7 | 78.6 ± 3.4 | 70.3 ± 4.5 | 68.9 ± 3.9 | 97.1 ± 10.0 |
| 3 | 6 | 55.3 ± 3.9 | 86.3 ± 27.4 | 87.9 ± 5.8 | 66.7 ± 4.4 | 76.3 ± 6.9 | 126.6 ± 13.6 |
| 4 | 6 | 62.4 ± 4.7 | 76.4 ± 7.3 | 88.4 ± 11.7 | 69.3 ± 2.3 | 72.6 ± 4.3 | 108.4 ± 28.6 |
| 5 | 6 | 59.1 ± 2.4 | 77.5 ± 7.3 | 82.7 ± 22.0 | 69.3 ± 1.1 | 71.3 ± 1.5 | 98.3 ± 5.3 |

Table 21. Comparison of Stability to Solubility Where at Least 50 % of the Solutions are Stable

| | Intact Soy pH 5 | |
|---|---|---|
| | 0.25% Xanthan Gum | |
| NaCl (%) | Stable (%) | Soluble (%) |
| 0 | 50 | 56 ± 18 |
| 1 | 100 | 82 ± 1 |
| 2 | 100 | 79 ± 3 |
| 3 | 83 | 88 ± 6 |
| 4 | 100 | 88 ± 12 |
| 5 | 67 | 83 ± 22 |

**Table22. Comparison of Stability to Solubility Where at Least 50% of the Solutions are Stable**

| | HYDROLYZED SOY pH 5 | | | |
|---|---|---|---|---|
| | 0.15% Xanthan Gum | | 0.25% Xanthan Gum | |
| NaCl (%) | Stable (%) | Soluble (%) | Stable (%) | Soluble (%) |
| 0 | 50 | 68 ± 5 | 50 | 104 ± 6 |
| 1 | 67 | 74 ± 6 | 83 | 78 ± 15 |
| 2 | 67 | 69 ± 4 | 67 | 97 ± 10 |
| 3 | 50 | 67 ± 4 | 67 | 127 ± 14 |
| 4 | * | ** | 50 | 108 ± 29 |
| 5 | * | ** | * | ** |

* Less than 50% Stable

** Not applicable

solutions at pH 5 had over 50% soluble protein. A comparison of intact soy solutions at pH 5 with 0.15% xanthan gum and those with 0.25% xanthan gum indicates they are remarkably similar as regards percent soluble protein. However, Table 21 indicates that at pH 5 only those intact soy solutions with 0.25% xanthan gum were greater than 50% stable. It is hypothesized that it is the insoluble soy protein components that bind, via hydrogen bonding, to the xanthan gum structure. It is hypothesized that at 0.15% xanthan gum there was not enough gum to bind the insoluble soy components. Therefore, precipitation occurred. Table 21 indicates that there was a positive relationship between the percentage of solutions visually stable and the percentage of soluble protein at 0.25% xanthan gum. The correlation coefficient was 0.71.

Looking again at Table 20 it can be seen that the majority of the hydrolyzed soy solutions at pH 5 had greater than 60% soluble protein. Compared to the intact soy solutions, the hydrolyzed soy protein solutions at pH 5 were higher in percent soluble protein. This is to be expected since the molecules of hydrolyzed soy are smaller than those of intact soy.

Table 22 indicates that hydrolyzed soy protein solutions at pH 5 were relatively stable when mixed with both 0.15 and 0.25% xanthan gum. This indicates that the

insoluble soy components may have complexed with the xanthan gum, thereby preventing their precipitation. For soy protein - 0.15% xanthan gum solutions at pH 5 the correlation coefficient between stability and percent soluble protein was 0.74. It should be noted, however, that there was little correlation between the percent stability and percent soluble protein for solutions of hydrolyzed soy protein - 0.25% xanthan gum. The correlation coefficient was -0.51. The interaction between proteins and xanthan gum are dynamic, forming an equilibrium between soluble and insoluble complexes. The apparent loss of correlation as the xanthan gum was increased from 0.15% to 0.25% may indicate these dynamics. It is perhaps possible that if the solubility was measured at a different point in time, the results might have been different.

The percent soluble protein in soy protein - 0.25% xanthan gum solutions with 3% added sodium chloride at pH's 3, 4, and 9 was also determined. The results are summarized in Table 23 . Percent soluble protein at pH 5 and pH 7 are shown for comparison. It can be seen that for intact soy the percent soluble protein ranged from 67.7 to 132.0%. At the extremes, pH 3 and pH 9, solubility was the lowest. Percent solubility could not be determined for intact soy - xanthan gum solutions at

**Table23. Mean Percent Soluble Protein in Soy Protein - 0.25% Xanthan Gum Solutions , 3% NaCl**

| pH | Intact | Hydrolyzed |
|---|---|---|
| 2 | * | 100.3 ± 17.0 |
| 3 | 67.7 ± 2.1 | 92.3 ± 7.4 |
| 4 | 103.7 ± 4.9 | 114.2 ± 6.5 |
| 5 | 88.0 ± 6.0 | 127.0 ± 14.0 |
| 7 | 132.0 ± 7.0 | 101.0 ± 4.0 |
| 9 | 68.8 ± 1.7 | 95.1 ± 4.7 |

* Indeterminable due to precipitation

pH 2, due to gross precipitation. Soy proteins become increasingly insoluble as the pH drops from pH 7.0 to pH 4.2 (Seal, 1980). It should be noted that with the exception of pH 9 the percent soluble protein appeared to be related to visual stability. If pH 9 is excluded, the correlation coefficient between stability and percent soluble protein was 0.90. Including pH 9, the correlation coefficient was 0.54. At pH 9, the solutions appeared 100% stable; however, some of the protein was insoluble. It is proposed that insoluble soy proteins remained suspended due to the solution viscosity.

Table 23 also summarizes the results of percent soluble protein for hydrolyzed soy - 0.25% xanthan gum with 3% added sodium chloride at pH's 2, 3, 4, 5, 7, and 9. Here the solubility ranged from 92.3 to 127.0%. The range of percent soluble protein in hydrolyzed soy solutions was less than the range in intact soy solutions. A correlation coefficient of -0.60 indicates a negative relationship between visual stability and percent soluble protein. Because hydrolyzed soy proteins are smaller than intact soy proteins, they might be expected to be more soluble.

Tables 24 and 25 summarize the results of percent soluble protein in solutions made with intact whey protein at pH's 7 and 5. For solutions with whey protein, solubility was determined only for solutions

**Table 24. Comparison of Stability to Solubility Where at Least 50% of the Solutions are Stable**

| | Intact Whey pH 7 | |
|---|---|---|
| | 0.25% Xanthan Gum | |
| NaCl (%) | Stable (%) | Soluble (%) |
| 0 | * | ** |
| 1 | 100 | 63 ± 4 |
| 2 | 66 | 66 ± 2 |
| 3 | 100 | 66 ± 4 |
| 4 | 100 | 66 ± 4 |
| 5 | 66 | 61 ± 5 |

* Less than 50% Stable

** Not Applicable

Table 25 . Mean Percentage of Soluble Protein in Whey Protein - 0.25% Xanthan Gum Solutions at pH 5

| | INTACT WHEY pH 5 | | | |
|---|---|---|---|---|
| | 0.15 % Xanthan Gum | | 0.25 % Xanthan Gum | |
| NaCl (%) | Stable (%) | Soluble (%) | Stable (%) | Soluble (%) |
| 0 | * | ** | * | ** |
| 1 | 100 | 63 ± 8 | * | ** |
| 2 | 100 | 59 ± 2 | 100 | 72 ± 9 |
| 3 | 66 | 57 ± 5 | 66 | 77 ± 4 |
| 4 | * | ** | 100 | 89 ± 4 |
| 5 | * | ** | 100 | 91 ± 7 |

* Less than 50 % Stable

** Not Applicable

that were greater than 50% stable. No whey protein solutions with 0.05 or 0.15% xanthan gum were stable at pH 7. At pH 7, 0.25% xanthan gum and 1 to 5% added sodium chloride were needed to stabilize the whey protein solutions. In Table 24, it can be seen that there was little relationship between stability and percent soluble protein. The correlation coefficient was only 0.36.

Table 25 indicates that for whey protein solutions at pH 5, with 0.15% xanthan gum there was a positive relationship between stability and percent soluble protein. Results indicated a correlation of 0.76. At 0.15% xanthan gum, the only whey protein solutions that were stable were those with 1 to 3 % added sodium chloride. At 4 and 5% added sodium chloride, it appeared the protein was salted - out of solution. While the whey protein - 0.15% xanthan gum solutions with 1 to 3% added sodium chloride were visually stable, it should be noted that they had from 37 to 43% insoluble protein. It is proposed that the insoluble proteins were held in suspension due to the solution viscosity and thus were not seen as precipitate.

Increasing the gum level to 0.25% in whey protein solutions at pH 5, resulted in more stable solutions than were seen at 0.15% xanthan gum. However, there was less correlation between percent visually stable and percent

soluble protein. Here the correlation coefficient was 0.38. It should also be noted from Table 25 that the whey protein -0.25% xanthan gum solutions at pH 5 required from 2 to 5 % added sodium chloride to remain stable. Higher concentrations of sodium chloride, such as 2 to 5%, would be expected to salt whey protein out of solution. By bonding to the available xanthan gum, the whey was protected from the effects of the sodium chloride. It is perhaps the ionic environment created by the anionic residues of xanthan gum that required at least 2% sodium chloride to be present for the whey protein to be capable of binding to the xanthan gum. It is proposed that whey protein and xanthan gum may interact such that an equilibrium between soluble and insoluble complexes is established. This equilibrium between soluble and insoluble complexes resulted in an apparent loss of correlation between stability and percent soluble protein when gum levels were increased from 0.15 to 0.25%.

## E. CHROMOTOGRAPHY

Elution profiles were successfully obtained for solutions of intact soy protein, with and without sodium chloride. Figure 20 illustrates the elution profile obtained from 2% soy protein solutions with 3% added sodium chloride. The profiles were typical of those for soy protein isolates as depicted by Hagegawa et al.,(1963). The 11S proteins came off the column first and were followed by the 7S fractions. It is interesting to note that the 11S soy protein fraction came off in the void volume of the column. The 11S soy protein fractions have a molecular weight of 350,000 (Wolf et al.,1959). The Sepharose CL-6B has a range from 10,000 to 4,000,000; therefore, it would not be expected that the 11S soy fraction would be eluted in the void volume. Wolf and Cowan (1975) have concluded that it is possible for the soy protein 11S fractions to form disulfide - linked polymers which are responsible for insolubility of soy protein. It is proposed that polymerization of the 11S fractions might have occurred, thus increasing the molecular weight such that this fraction would be eluted in the void volume. The addition of 3% sodium chloride did not alter the profile from that seen for soy protein isolate without added sodium chloride.

Successful fractionation of the 2% soy solutions, without xanthan gum, indicated that the gel filtration column was functioning properly. Despite this, attempts to

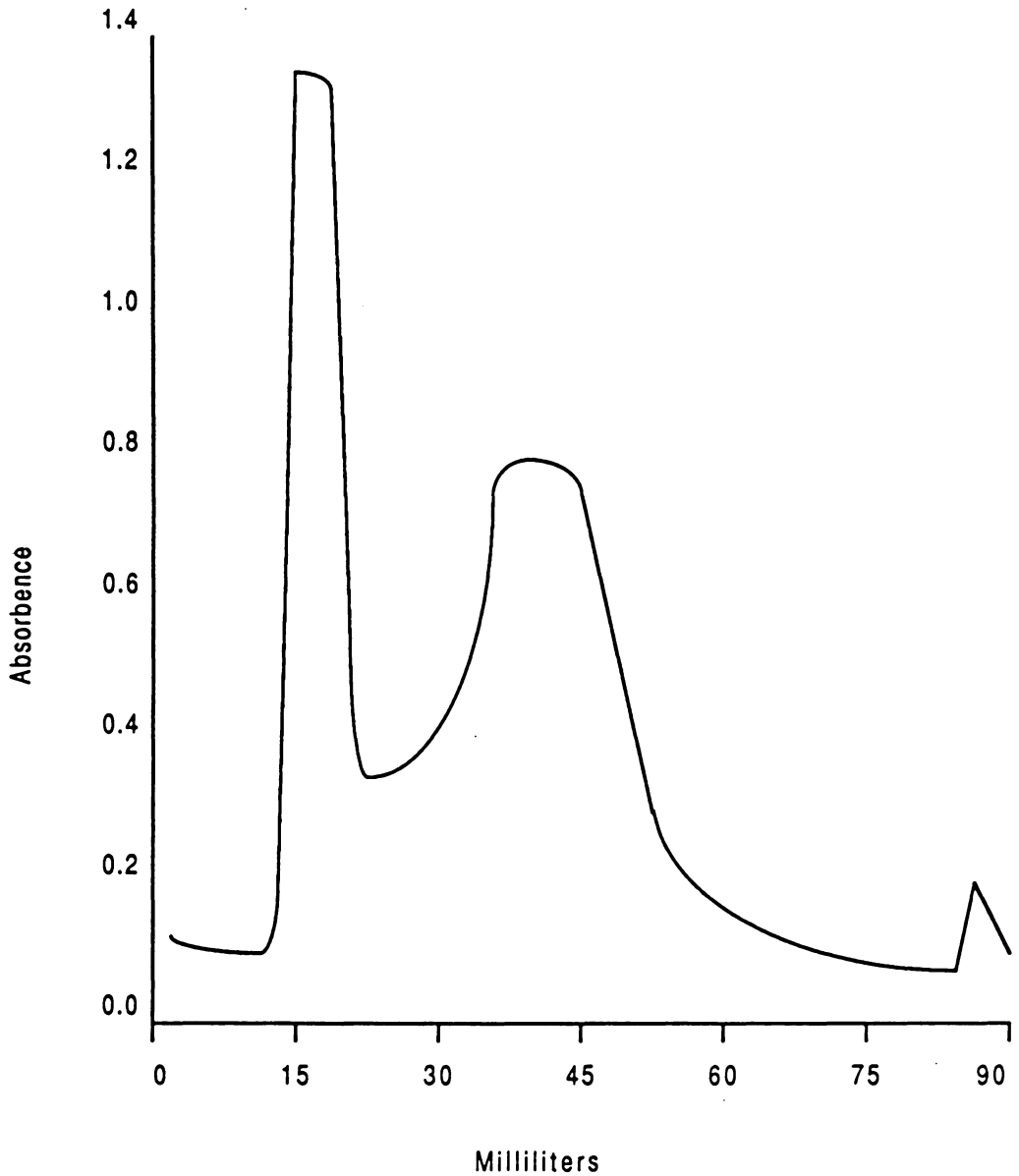


**Figure 20.. Elution Profile of 2% Intact Soy Protein Isolate, 3% Sodium Chloride Solution, pH 7 at 280 nm.**

run a solution of 0.25% xanthan gum through the column were unsuccessful. Figure 21 illustrates the elution profile obtained from a solution of 0.25% xanthan gum. From Figure 21 it can be seen that the majority of the xanthan gum came off the column at 30 milliliters . The void volume was approximately 20 milliliters. Therefore, the gum did not come off in the void volume, despite its large molecular weight. Soy proteins have a globular configuration while xanthan gum has a helical configuration. It is this difference that perhaps explains why the xanthan gum did not come off the column in the void volume while the soy protein did. Movement of a molecule through the column is dependent both on molecular size and molecular configuration. Measuring xanthan gum recovery using the phenol - sulfuric method indicated incomplete recovery. Figure 22 illustrates the standard curve obtained using the Phenol Sulfuric Method for quantifying xanthan gum.

Column chromotography may not be a viable tool for analysis of soy protein - xanthan gum solutions because the solutions are viscous. Another instrument that might be tried is a differential scanning calorimeter.

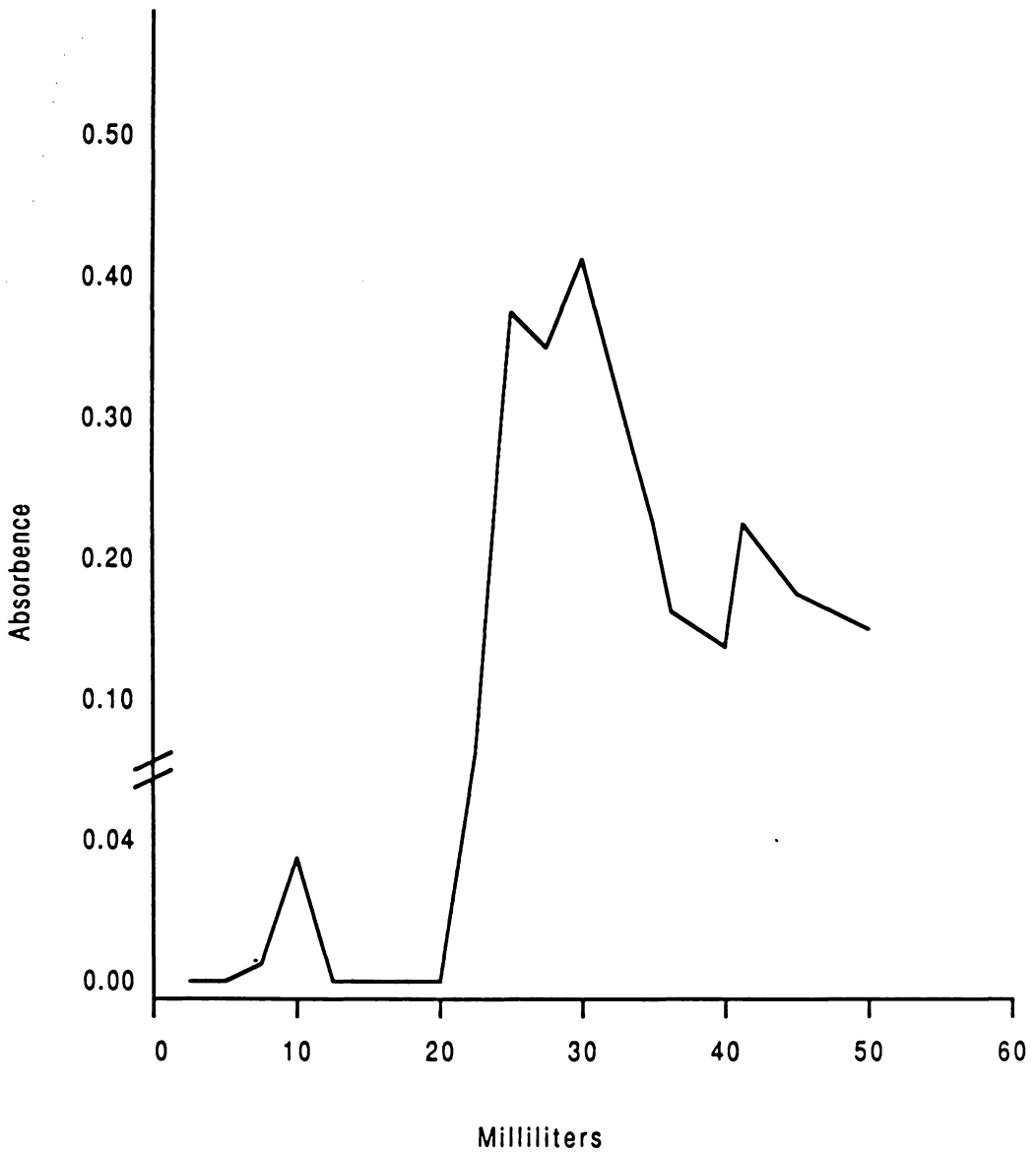


**Figure 21. Elution Profile of 0.25% Xanthan Gum Solution, at 490 nm.**

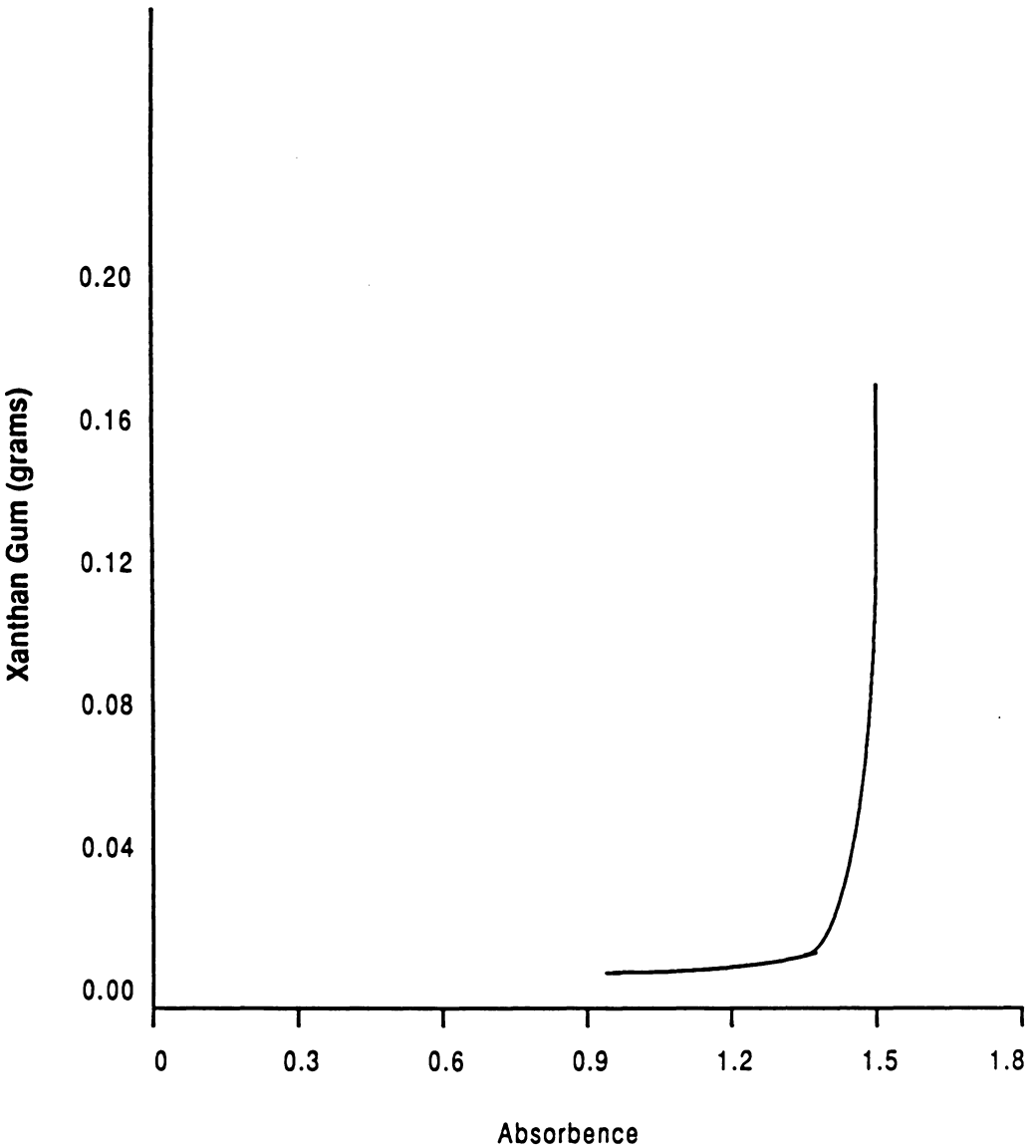


**Figure 22. Standard Curve for Phenol-Sulfuric Determination of Xanthan Gum.**

# IV. SUMMARY AND CONCLUSION

Research has led to the conclusion that when mixed with up to 0.25% xanthan gum, both soy sauce and tamari sauce formed stable, homogenous solutions. The addition of xanthan gum to soy and tamari sauce resulted in an obvious viscosity synergism, which was most marked in the tamari sauce-xanthan gum solutions. Increasing the xanthan gum concentration from 0.15% to 0.25% in these sauces resulted in an increase in pseudoplasticity.

It was concluded that through an adaptation of the Hsu et al. (1977) method of protein digestion, protease could be used on soy protein isolate to simulate the hydrolyzed soy protein present in soy sauce and tamari sauce. The use of hydrolyzed soy protein isolates in model systems indicated that the structure of the soy protein did affect the soy protein-xanthan gum interactions.

In general, it was concluded that the degree of hydrogen bonding between soy protein and xanthan gum is what influenced solution stability. Some hydrogen bonding appeared to result in the formation of soluble soy protein-xanthan gum complexes. Excessive hydrogen bonding appeared to result in the formation of insoluble protein-xanthan gum complexes, which led in turn to precipitation. Hence, it was proposed that there exists an optimal degree of soy protein-xanthan gum bonding, above or below which

precipitation results.

Factors that appeared to affect the degree of protein to xanthan gum bonding were protein structure, pH, gum concentration, and sodium chloride concentration.

Whey protein-xanthan gum solutions were similar to soy protein-xanthan gum solutions in that solution stability was affected by pH, gum concentration, and sodium chloride concentration. Solutions of whey protein concentrate at pH's 5 and 7 precipitated when mixed with xanthan gum alone, with 0% added sodium chloride. It was concluded that combinations of xanthan gum and sodium chloride whereby optimal stability was obtained. Altering these combinations resulted in precipitation of the whey protein.

Several conclusions were made regarding soy protein-xanthan gum solution solubility. One conclusion was that increasing sodium chloride concentation resulted in increasing solubility. It was however further concluded that the addition of excessive salt resulted in decreased solubility. What constituted excessive sodium chloride depended on the combination of pH, protein type, and gum concentration.

It was noted that increasing xanthan gum concentration resulted in increasing protein solubility. Results indicated that hydrolyzed soy protein-xanthan gum solutions were more soluble than similar solutions of intact soy protein-xanthan gum. At pH extremes of 2, 3, and 9, there was a loss of

solubility in the soy protein-0.25% xanthan gum solutions with 3% added sodium chloride.

Visually determined stability did not necessarily correlate with the percentage of soluble protein. Large standard deviations noted in the percent soluble protein suggested that an equilibrium might exist between soluble aggregates and inoluble aggregates. All results indicated that the interactions between soy or whey proteins and xanthan gum were dynamic ones. It appeared that a equilibrium existed whereby protein-xanthan gum bonds were established, broken, and perhaps reestablished.

Gel filtration as a method of fractionating soy protein-xanthan gum solutions was unsuccessful. The lack of success was due to the relatively high viscosity of the soy protein-xanthan gum solutions.

# V. SUGGESTIONS FOR FURTHER RESEARCH

The research has indicated that a dynamic equilibrium exists between protein and xanthan gum. Future work regarding protein - xanthan gum interactions should examine the kinetics of the interaction. This might be accomplished by measuring the percent soluble protein in a solution at various intervals throughout a forty - eight hour interval.

Studying the reaction of xanthan gum with proteins other than soy and whey should also be informative. The method used for evaluation of the protein - xanthan gum solutions should be consistent, so that valuable comparisons could be made between protein types. To date, the methodology used to study protein - polysaccharide interactions have not been consistent; thereby making comparisons difficult.

Another recommendation for further research is to evaluate the effects of various types of salts or protein - xanthan gum solutions. Specifically, calcium chloride and potassium chloride should be evaluated.

Future work should focus on defining the composition of the precipitates formed; is the precipitate composed of protein only, or is it a combination of protein and gum? This information might be obtained by centrifugation of protein - xanthan gum solutions, followed by evaluation of the precipitate. The current research evaluated only the supernatant.

In food applications, protein - polysaccharide solutions are often exposed to heat. It is suggested that the effects of heating model protein -polysaccharide solutions be examined.

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## VII. APPENDIX A

Viscosity Readings of Intact Soy Protein - Xanthan Gum Solutions at pH 7

| XG (%) | NaCl (%) | RPM 2.5 | 5 | 10 | 20 | 50 | 100 |
|---|---|---|---|---|---|---|---|
| 0.05 | 3 | 5.12 | 6.40 | 6.40 | 6.08 | 4.80 | 3.68 |
| 0.05 | 4 | 6.40 | 7.68 | 6.40 | 6.40 | 4.99 | 4.10 |
| 0.05 | 5 | 6.40 | 6.40 | 6.40 | 6.08 | 5.12 | 4.96 |
| 0.15 | 1 | 140.79 | 99.20 | 67.84 | 46.08 | 27.65 | 18.56 |
| 0.15 | 1 | 138.24 | 96.00 | 66.56 | 45.12 | 26.88 | 18.94 |
| 0.15 | 2 | 143.39 | 99.20 | 68.80 | 46.08 | 27.52 | 19.10 |
| 0.15 | 3 | 96.00 | 67.84 | 48.00 | 33.12 | 19.84 | 13.92 |
| 0.15 | 3 | 176.64 | 117.75 | 79.35 | 52.80 | 31.23 | 21.12 |
| 0.15 | 3 | 159.99 | 111.35 | 74.24 | 49.60 | 29.44 | 20.32 |
| 0.15 | 4 | 211.20 | 133.10 | 80.63 | 49.60 | 29.76 | 19.58 |
| 0.15 | 4 | 110.10 | 78.10 | 54.40 | 36.48 | 21.63 | 14.53 |
| 0.15 | 4 | 160.00 | 110.70 | 74.24 | 50.40 | 29.76 | 20.54 |
| 0.15 | 4 | 160.00 | 110.70 | 72.96 | 49.60 | 29.57 | 20.54 |
| 0.15 | 4 | 153.61 | 108.20 | 72.96 | 48.32 | 28.67 | 19.84 |
| 0.15 | 4 | 163.84 | 107.52 | 72.96 | 48.80 | 28.80 | 19.84 |
| 0.15 | 5 | 112.64 | 80.60 | 56.32 | 38.08 | 22.53 | 15.68 |
| 0.15 | 5 | 128.00 | 90.90 | 62.72 | 42.88 | 25.47 | 16.96 |
| 0.15 | 5 | 147.20 | 105.00 | 70.40 | 48.00 | 28.48 | 19.84 |
| 0.15 | 5 | 162.55 | 110.70 | 74.88 | 50.40 | 29.44 | 19.90 |
| 0.15 | 5 | 273.91 | 176.60 | 112.64 | 71.68 | 40.32 | 25.92 |
| 0.25 | 1 | 284.15 | 185.60 | 121.60 | 76.80 | 45.76 | 27.01 |
| 0.25 | 1 | 1.00 | 259.82 | 163.84 | 108.48 | 59.52 | 36.16 |
| 0.25 | 2 | 473.62 | 303.99 | 180.80 | 114.56 | 68.48 | 38.56 |
| 0.25 | 2 | 427.52 | 270.08 | 180.48 | 121.28 | 57.28 | 36.10 |
| 0.25 | 2 | 424.96 | 266.24 | 175.70 | 105.92 | 58.75 | 36.96 |
| 0.25 | 3 | 394.26 | 248.32 | 159.37 | 104.64 | 57.28 | 36.22 |
| 0.25 | 3 | 432.68 | 270.08 | 170.24 | 105.60 | 57.73 | 36.93 |
| 0.25 | 3 | 437.77 | 272.63 | 172.79 | 113.92 | 58.56 | 37.18 |
| 0.25 | 3 | 458.24 | 294.39 | 175.35 | 110.72 | 60.42 | 37.44 |
| 0.25 | 3 | 448.00 | 257.29 | 166.40 | 105.28 | 57.28 | 36.16 |
| 0.25 | 3 | 450.56 | 282.90 | 182.40 | 110.72 | 59.65 | 37.12 |

APPENDIX A continued

| XG (%) | NaCl (%) | RPM 2.5 | 5 | 10 | 20 | 50 | 100 |
|---|---|---|---|---|---|---|---|
| 0.25 | 4 | 399.37 | 249.61 | 159.05 | 100.00 | 54.40 | 34.94 |
| 0.25 | 4 | 471.02 | 293.10 | 179.20 | 113.28 | 60.35 | 37.92 |
| 0.25 | 4 | 441.60 | 277.80 | 171.19 | 106.88 | 59.20 | 36.10 |
| 0.25 | 4 | 455.68 | 280.31 | 175.35 | 297.50 | 59.14 | 37.28 |
| 0.25 | 4 | 432.64 | 281.60 | 174.08 | 110.72 | 60.29 | 36.61 |
| 0.25 | 4 | 437.77 | 280.31 | 174.72 | 108.80 | 59.65 | 36.64 |
| 0.25 | 5 | 383.98 | 259.20 | 155.52 | 99.10 | 57.86 | 33.12 |
| 0.25 | 5 | 435.20 | 268.81 | 172.79 | 109.11 | 59.52 | 36.26 |
| 0.25 | 5 | 454.41 | 308.49 | 176.96 | 110.08 | 58.88 | 37.44 |
| 0.25 | 5 | 445.46 | 285.40 | 174.08 | 110.08 | 58.88 | 37.12 |
| 0.25 | 5 | 518.38 | 288.59 | 178.56 | 111.21 | 60.16 | 34.88 |
| 0.25 | 5 | 435.20 | 268.81 | 170.24 | 108.48 | 58.00 | 36.79 |

# APPENDIX B

Viscosity Readings of Hydrolyzed Soy Protein - Xanthan Gum Solutions at pH 7 and 25 C.

| XG (%) | NaCl (%) | RPM 2.5 | 5 | 10 | 20 | 50 | 100 |
|---|---|---|---|---|---|---|---|
| 0.05 | 2 | 12.80 | 12.80 | 11.20 | 9.28 | 7.36 | 5.60 |
| 0.05 | 2 | 15.36 | 12.80 | 11.20 | 8.64 | 7.17 | 5.57 |
| 0.05 | 3 | 15.36 | 15.36 | 12.80 | 11.20 | 8.06 | 6.46 |
| 0.05 | 3 | 23.04 | 20.48 | 16.64 | 12.50 | 9.09 | 8.90 |
| 0.05 | 3 | 23.04 | 20.48 | 16.00 | 12.50 | 9.08 | 8.00 |
| 0.05 | 3 | 23.04 | 19.20 | 16.00 | 12.50 | 9.09 | 7.94 |
| 0.05 | 4 | 19.20 | 19.20 | 15.36 | 12.80 | 9.09 | 8.00 |
| 0.05 | 5 | 15.36 | 14.08 | 12.16 | 6.40 | 7.81 | 5.25 |
| 0.05 | 5 | 5.25 | 20.48 | 17.28 | 13.60 | 9.60 | 9.12 |
| 0.15 | 1 | 130.56 | 92.16 | 62.40 | 42.88 | 25.09 | 17.22 |
| 0.15 | 2 | 115.20 | 83.20 | 57.60 | 39.40 | 23.68 | 16.48 |
| 0.15 | 2 | 138.24 | 96.00 | 65.60 | 43.52 | 26.24 | 17.66 |
| 0.15 | 2 | 117.75 | 84.48 | 57.60 | 39.68 | 23.94 | 16.06 |
| 0.15 | 2 | 134.40 | 92.80 | 64.64 | 43.52 | 25.73 | 17.41 |
| 0.15 | 3 | 140.79 | 97.28 | 66.56 | 44.60 | 26.50 | 16.16 |
| 0.15 | 3 | 128.00 | 90.88 | 62.40 | 42.24 | 25.09 | 17.79 |
| 0.15 | 3 | 143.37 | 101.10 | 68.48 | 46.08 | 27.20 | 18.40 |
| 0.15 | 3 | 168.97 | 108.80 | 70.40 | 48.80 | 28.29 | 19.07 |
| 0.15 | 3 | 163.84 | 105.60 | 71.68 | 47.68 | 28.03 | 18.72 |
| 0.15 | 3 | 153.61 | 107.52 | 72.00 | 48.00 | 28.03 | 18.94 |
| 0.15 | 4 | 112.64 | 80.64 | 56.00 | 38.40 | 23.04 | 15.68 |
| 0.15 | 4 | 145.93 | 99.84 | 67.84 | 45.60 | 26.88 | 18.43 |
| 0.15 | 4 | 134.40 | 94.72 | 64.64 | 43.52 | 26.24 | 18.18 |
| 0.15 | 4 | 135.68 | 94.72 | 64.64 | 43.52 | 25.60 | 17.67 |
| 0.15 | 4 | 151.03 | 105.60 | 70.40 | 47.36 | 28.03 | 18.82 |
| 0.15 | 4 | 156.16 | 105.60 | 71.68 | 48.00 | 28.29 | 18.88 |
| 0.15 | 5 | 143.37 | 99.84 | 68.48 | 45.60 | 26.75 | 18.62 |
| 0.15 | 5 | 133.13 | 90.88 | 61.44 | 42.24 | 24.96 | 16.48 |
| 0.15 | 5 | 147.20 | 102.40 | 69.12 | 46.08 | 27.78 | 18.62 |
| 0.15 | 5 | 166.40 | 115.20 | 76.16 | 50.56 | 29.95 | 20.80 |
| 0.15 | 5 | 160.00 | 110.08 | 74.24 | 49.60 | 28.80 | 19.39 |
| 0.15 | 5 | 166.40 | 115.20 | 76.80 | 51.20 | 29.44 | 20.37 |
| 0.25 | 1 | 345.61 | 243.20 | 144.00 | 91.52 | 51.20 | 32.64 |

APPENDIX B continued

| XG (%) | NaCl (%) | RPM 2.5 | 5 | 10 | 20 | 50 | 100 |
|---|---|---|---|---|---|---|---|
| 0.25 | 2 | 351.99 | 223.99 | 147.20 | 93.12 | 54.08 | 32.00 |
| 0.25 | 2 | 394.26 | 252.80 | 158.40 | 98.40 | 56.00 | 34.30 |
| 0.25 | 2 | 373.75 | 248.32 | 157.43 | 102.40 | 56.83 | 33.60 |
| 0.25 | 3 | 422.42 | 264.97 | 165.75 | 102.08 | 55.68 | 34.25 |
| 0.25 | 3 | 399.37 | 247.05 | 156.80 | 97.60 | 55.04 | 33.12 |
| 0.25 | 3 | 383.98 | 244.47 | 152.32 | 103.68 | 53.25 | 34.11 |
| 0.25 | 3 | 445.46 | 272.65 | 173.43 | 107.20 | 57.60 | 36.74 |
| 0.25 | 4 | 368.63 | 245.77 | 149.75 | 93.60 | 50.63 | 32.48 |
| 0.25 | 4 | 404.48 | 276.47 | 160.65 | 99.20 | 52.16 | 33.22 |
| 0.25 | 4 | 396.79 | 263.67 | 156.80 | 97.60 | 53.76 | 33.09 |
| 0.25 | 4 | 404.48 | 263.67 | 168.01 | 110.40 | 59.52 | 35.52 |
| 0.25 | 5 | 417.30 | 259.85 | 162.57 | 103.05 | 56.58 | 33.79 |
| 0.25 | 5 | 437.77 | 278.41 | 176.64 | 103.68 | 56.58 | 35.46 |
| 0.25 | 5 | 437.77 | 323.21 | 174.08 | 106.88 | 57.60 | 36.29 |
| 0.25 | 5 | 445.46 | 272.65 | 173.43 | 107.20 | 57.60 | 36.74 |

# APPENDIX C

## Viscosity Readings of Intact Soy Protein - Xanthan Gum Solutions at pH 5

| XG (%) | NaCl (%) | RPM 2.5 | 5 | 10 | 20 | 50 | 100 |
|---|---|---|---|---|---|---|---|
| 0.15 | 0 | 108.80 | 76.80 | 53.76 | 37.76 | 24.06 | 17.92 |
| 0.15 | 0 | 87.04 | 65.28 | 47.36 | 33.92 | 22.02 | 16.32 |
| 0.15 | 1 | 147.20 | 102.40 | 69.12 | 47.04 | 28.03 | 19.14 |
| 0.15 | 2 | 171.52 | 120.33 | 83.20 | 56.32 | 33.28 | 22.72 |
| 0.15 | 3 | 161.27 | 112.00 | 76.80 | 52.80 | ERR | 21.76 |
| 0.15 | 4 | 181.76 | 124.80 | 85.76 | 57.28 | 34.05 | 23.17 |
| 0.15 | 5 | 211.20 | 134.40 | 90.24 | 60.80 | 35.84 | 23.81 |
| 0.25 | 0 | 358.38 | 226.56 | 143.75 | 92.16 | 50.69 | 33.12 |
| 0.25 | 0 | 314.88 | 198.40 | 127.36 | 80.64 | 45.44 | 29.60 |
| 0.25 | 0 | 343.02 | 222.72 | 144.63 | 93.44 | 50.82 | 33.02 |
| 0.25 | 1 | 505.58 | 357.13 | 201.60 | 124.47 | 68.61 | 44.03 |
| 0.25 | 1 | 529.96 | 334.09 | 207.99 | 130.40 | 69.89 | 45.18 |
| 0.25 | 1 | 486.38 | 305.91 | 195.20 | 120.00 | 64.90 | 41.44 |
| 0.25 | 1 | 488.97 | 326.39 | 205.45 | 131.53 | 68.86 | 43.71 |
| 0.25 | 1 | 509.43 | 321.28 | 199.04 | 125.44 | 68.99 | 42.62 |
| 0.25 | 1 | 527.37 | 327.67 | 203.53 | 128.00 | 68.61 | 43.58 |
| 0.25 | 2 | 532.46 | 337.92 | 212.47 | 133.13 | 71.81 | 44.86 |
| 0.25 | 2 | 496.66 | 316.81 | 200.96 | 126.72 | 67.97 | 43.20 |
| 0.25 | 2 | 445.46 | 282.87 | 183.68 | 114.24 | 61.57 | 38.78 |
| 0.25 | 2 | 518.38 | 327.67 | 211.20 | 129.28 | 69.25 | 42.75 |
| 0.25 | 2 | 537.59 | 346.89 | 220.81 | 136.96 | 71.81 | 45.92 |
| 0.25 | 2 | 518.38 | 323.21 | 204.16 | 128.00 | 70.02 | 42.94 |
| 0.25 | 3 | 488.97 | 304.63 | 193.27 | 119.20 | 65.02 | 40.32 |
| 0.25 | 3 | 524.79 | 340.46 | 213.77 | 133.61 | 71.42 | 45.12 |
| 0.25 | 3 | 552.97 | 345.61 | 215.03 | 140.16 | 71.17 | 45.06 |
| 0.25 | 3 | 448.00 | 280.31 | 180.48 | 112.75 | 60.67 | 38.08 |
| 0.25 | 3 | 552.97 | 344.33 | 213.77 | 135.04 | 71.68 | 44.54 |

APPENDIX C continued

| XG (%) | NaCl (%) | RPM 2.5 | 5 | 10 | 20 | 50 | 100 |
|---|---|---|---|---|---|---|---|
| 0.25 | 4 | 569.58 | 353.29 | 220.81 | 136.96 | 72.70 | 45.25 |
| 0.25 | 4 | 578.54 | 377.59 | 250.89 | 141.60 | 77.95 | 48.51 |
| 0.25 | 4 | 522.23 | 323.21 | 200.96 | 124.00 | 65.15 | 42.24 |
| 0.25 | 4 | 499.20 | 314.88 | 195.84 | 122.56 | 66.43 | 41.34 |
| 0.25 | 4 | 537.59 | 336.64 | 208.64 | 130.56 | 69.63 | 43.20 |
| 0.25 | 4 | 550.38 | 341.76 | 212.47 | 132.79 | 70.91 | 42.05 |
| 0.25 | 5 | 552.97 | 342.41 | 213.77 | 134.08 | 72.32 | 44.54 |
| 0.25 | 5 | 449.21 | 314.88 | 195.20 | 120.33 | 64.00 | 40.64 |
| 0.25 | 5 | 573.47 | 358.38 | 223.99 | 138.39 | 72.45 | 44.32 |
| 0.25 | 5 | 504.31 | 319.99 | 198.40 | 125.44 | 67.07 | 41.60 |

# APPENDIX D

Viscosity Readings of Hydroyzed Soy Protein - Xanthan Gum Solutions at pH 5.

| XG (%) | NaCl (%) | RPM 2.5 | 5 | 10 | 20 | 50 | 100 |
|---|---|---|---|---|---|---|---|
| 0.05 | 0 | 15.36 | 15.36 | 12.80 | 10.88 | 7.94 | 6.72 |
| 0.05 | 0 | 15.36 | 15.36 | 12.00 | 20.00 | 50.00 | 100.00 |
| 0.05 | 1 | 15.36 | 16.64 | 13.44 | 11.20 | 8.45 | 8.26 |
| 0.05 | 1 | 17.92 | 14.08 | 13.44 | 10.88 | 7.81 | 5.40 |
| 0.05 | 2 | 17.92 | 16.64 | 13.44 | 10.88 | 8.32 | 8.06 |
| 0.05 | 3 | 17.92 | 16.64 | 13.44 | 10.88 | 8.32 | 7.23 |
| 0.05 | 3 | 20.48 | 16.00 | 12.80 | 11.20 | 8.45 | 8.00 |
| 0.05 | 4 | 20.48 | 19.20 | 15.36 | 11.84 | 8.96 | 6.91 |
| 0.15 | 0 | 148.49 | 102.40 | 70.40 | 43.84 | 28.42 | 19.97 |
| 0.15 | 0 | 145.93 | 101.12 | 69.12 | 46.72 | 27.78 | 17.09 |
| 0.15 | 0 | 140.79 | 99.20 | 69.12 | 47.04 | 28.03 | 19.30 |
| 0.15 | 1 | 153.61 | 104.96 | 70.40 | 47.04 | 28.03 | 18.11 |
| 0.15 | 1 | 148.49 | 103.68 | 69.76 | 46.72 | 27.65 | 19.20 |
| 0.15 | 1 | 151.03 | 104.96 | 71.04 | 47.68 | 28.16 | 19.30 |
| 0.15 | 1 | 147.20 | 102.40 | 69.12 | 47.04 | 28.03 | 19.10 |
| 0.15 | 2 | 166.40 | 110.08 | 72.96 | 49.28 | 29.31 | 19.52 |
| 0.15 | 2 | 148.49 | 103.68 | 69.76 | 47.04 | 28.16 | 18.88 |
| 0.15 | 2 | 153.61 | 107.52 | 72.96 | 48.00 | 28.29 | 19.26 |
| 0.15 | 2 | 151.03 | 104.96 | 70.40 | 47.04 | 27.90 | 18.50 |
| 0.15 | 3 | 163.84 | 111.36 | 74.88 | 51.20 | 30.72 | 21.12 |
| 0.15 | 3 | 163.84 | 110.08 | 73.60 | 48.96 | 29.06 | 19.90 |
| 0.15 | 3 | 176.64 | 115.20 | 72.32 | 49.92 | 29.44 | 20.54 |
| 0.15 | 4 | 156.16 | 106.24 | 71.68 | 48.00 | 28.29 | 19.70 |
| 0.15 | 5 | 168.97 | 115.20 | 76.80 | 51.84 | 30.34 | 20.61 |
| 0.15 | 5 | 168.97 | 115.20 | 76.80 | 51.84 | 30.34 | 20.61 |
| 0.15 | 5 | 166.40 | 115.20 | 77.40 | 51.52 | 30.21 | 21.06 |
| 0.25 | 0 | 414.72 | 264.97 | 169.59 | 114.88 | 60.67 | 36.54 |
| 0.25 | 0 | 427.52 | 271.35 | 170.89 | 105.92 | 54.78 | 36.30 |
| 0.25 | 0 | 394.26 | 253.43 | 160.00 | 101.12 | 56.83 | 37.60 |

APPENDIX D continued

| XG (%) | NaCl (%) | RPM 2.5 | 5 | 10 | 20 | 50 | 100 |
|---|---|---|---|---|---|---|---|
| 0.25 | 1 | 427.52 | 270.08 | 175.35 | 105.60 | 58.24 | 36.32 |
| 0.25 | 1 | 427.52 | 270.08 | 172.79 | 106.56 | 58.50 | 36.42 |
| 0.25 | 1 | 427.52 | 271.35 | 170.89 | 105.92 | 54.78 | 36.30 |
| 0.25 | 1 | 427.52 | 272.63 | 172.16 | 105.28 | 57.86 | 36.10 |
| 0.25 | 1 | 427.52 | 271.35 | 172.79 | 109.11 | 56.96 | 36.60 |
| 0.25 | 2 | 442.88 | 280.31 | 176.00 | 110.08 | 61.95 | 38.91 |
| 0.25 | 2 | 437.82 | 275.20 | 175.35 | 109.44 | 61.70 | 36.54 |
| 0.25 | 2 | 430.09 | 270.08 | 168.01 | 105.92 | 63.49 | 35.90 |
| 0.25 | 2 | 424.96 | 271.35 | 170.89 | 108.48 | 56.70 | 35.39 |
| 0.25 | 3 | 427.52 | 266.24 | 170.24 | 107.52 | 59.90 | 36.54 |
| 0.25 | 3 | 435.20 | 273.91 | 172.79 | 109.11 | 58.24 | 36.74 |
| 0.25 | 3 | 445.46 | 279.05 | 175.35 | 109.44 | 59.14 | 35.36 |
| 0.25 | 3 | 616.96 | 383.98 | 246.41 | 147.20 | 78.34 | 48.32 |
| 0.25 | 4 | 443.68 | 281.60 | 178.56 | 112.64 | 60.42 | 37.12 |
| 0.25 | 4 | 442.88 | 278.41 | 175.35 | 110.40 | * | * |
| 0.25 | 4 | 488.97 | 305.91 | 190.72 | 117.12 | 63.87 | 38.98 |
| 0.25 | 5 | 445.46 | 280.31 | 172.79 | 109.44 | 59.39 | 37.12 |
| 0.25 | 5 | 460.82 | 284.15 | 181.76 | 114.24 | 61.57 | 37.44 |

# APPENDIX E

Absorbance Readings and Percent Soluble Protein in Soy Protein - Xanthan Gum Solution where:

pH = 7
Gum = 0.05 %
Protein = Intact Soy

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | |
|---|---|---|---|---|
| | | 0% NaCl | | |
| 1 | 8.93 | 8.38 | 40.50 | |
| 2 | 9.72 | 9.17 | 44.32 | |
| 3 | 8.53 | 7.98 | 38.57 | |
| 4 | 8.76 | 8.21 | 39.68 | |
| | | | Mean % soluble protein: | 40.77 |
| | | 1% NaCl | | |
| 1 | 6.04 | 5.49 | 26.53 | |
| 2 | 7.25 | 6.70 | 32.38 | |
| 3 | 7.89 | 7.34 | 35.48 | |
| 4 | 6.25 | 5.70 | 27.55 | |
| | | | Mean % soluble protein: | 30.49 |
| | | 2% NaCl | | |
| 1 | 6.82 | 6.27 | 30.30 | |
| 2 | 6.31 | 5.76 | 27.84 | |
| 3 | 7.58 | 7.03 | 33.98 | |
| 4 | 6.52 | 5.97 | 28.85 | |
| | | | Mean % soluble protein: | 30.24 |
| | | 3% NaCl | | |
| 1 | 9.13 | 8.58 | 41.47 | |
| 2 | 10.43 | 9.88 | 47.75 | |
| 3 | 7.13 | 6.58 | 31.80 | |
| 4 | 8.43 | 7.88 | 38.09 | |
| | | | Mean % soluble protein: | 39.78 |
| | | 4% NaCl | | |
| 1 | 8.58 | 8.03 | 38.81 | |
| 2 | 7.11 | 6.56 | 31.71 | |
| 3 | 6.80 | 6.25 | 30.21 | |
| 4 | 7.04 | 6.49 | 31.37 | |

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein |
|---|---|---|---|
| | | 5% NaCl | |
| 1 | 8.58 | 8.03 | 38.81 |
| 2 | 6.03 | 5.48 | 26.49 |
| 3 | 6.44 | 5.89 | 28.47 |
| 4 | 6.77 | 6.22 | 30.06 |

Absorbance Readings and Percent Soluble Protein in Soy Protein - Xanthan Gum Solution where:

pH = 7
Gum = 0.15 %
Protein = Intact Soy

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | |
|---|---|---|---|---|
| | | 0% NaCl | | |
| 1 | 11.40 | 10.22 | 49.40 | |
| 2 | 13.24 | 11.20 | 54.13 | |
| 3 | 10.65 | 8.61 | 41.61 | |
| 4 | 10.30 | 8.26 | 39.92 | |
| 5 | 10.10 | 8.06 | 38.96 | |
| 6 | 10.78 | 8.74 | 42.24 | |
| | | | Mean % soluble protein: | 44.38 |
| | | 1% NaCl | | |
| 1 | 12.75 | 10.71 | 51.76 | |
| 2 | 10.34 | 8.30 | 40.12 | |
| 3 | 13.34 | 11.30 | 54.62 | |
| 4 | 11.50 | 9.46 | 45.72 | |
| 5 | 10.90 | 8.86 | 42.82 | |
| 6 | 11.34 | 9.30 | 44.95 | |
| | | | Mean % soluble protein: | 46.67 |
| | | 2% NaCl | | |
| 1 | 11.00 | 8.96 | 43.31 | |
| 2 | 18.21 | 16.17 | 78.15 | |
| 3 | 12.41 | 10.37 | 50.12 | |
| 4 | 11.49 | 9.45 | 45.67 | |
| 5 | 12.30 | 10.26 | 49.59 | |
| 6 | 11.90 | 9.86 | 47.66 | |
| | | | Mean % soluble protein: | 52.42 |
| | | 3% NaCl | | |
| 1 | 12.12 | 10.08 | 48.72 | |
| 2 | 11.74 | 9.70 | 46.88 | |
| 3 | 13.53 | 11.49 | 55.53 | |
| 4 | 11.60 | 9.56 | 46.21 | |
| 5 | 11.79 | 9.75 | 47.12 | |
| 6 | 12.20 | 10.16 | 49.11 | |
| | | | Mean % soluble protein: | 48.93 |
| | | 4% NaCl | | |
| 1 | 11.50 | 9.46 | 45.72 | |
| 2 | 12.16 | 10.12 | 48.91 | |
| 3 | 12.10 | 10.06 | 48.62 | |
| 4 | 12.50 | 10.46 | 50.56 | |
| 5 | 11.90 | 9.86 | 47.66 | |
| 6 | 12.25 | 10.21 | 49.35 | |
| | | | Mean % soluble protein: | 48.47 |
| | | 5% NaCl | | |
| 1 | 12.01 | 9.97 | 48.19 | |
| 2 | 13.54 | 11.50 | 55.58 | |
| 3 | 12.76 | 10.72 | 51.81 | |
| 4 | 12.50 | 10.46 | 50.56 | |
| 5 | 12.10 | 10.06 | 48.62 | |
| 6 | 13.25 | 11.21 | 54.18 | |
| | | | Mean % soluble protein: | 51.49 |

Absorbance Readings and Percent Soluble Protein in Soy Protein - Xanthan Gum Solutions where:

pH = 7
Gum = 0.25 %
Protein = Intact Soy

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | |
|---|---|---|---|---|
| | | 0% NaCl | | |
| 1 | 12.62 | 10.78 | 52.10 | |
| 2 | 12.50 | 10.46 | 50.56 | |
| 3 | 24.93 | 22.89 | 110.63 | |
| 4 | 13.20 | 11.16 | 53.94 | |
| 5 | 12.50 | 10.46 | 50.56 | |
| 6 | 12.60 | 10.76 | 52.01 | |
| | | | Mean % soluble protein: | 61.63 |
| | | 1% NaCl | | |
| 1 | 20.66 | 18.62 | 90.00 | |
| 2 | 16.06 | 14.02 | 67.76 | |
| 3 | 17.50 | 15.46 | 74.72 | |
| 4 | 15.50 | 13.46 | 65.06 | |
| 5 | 16.30 | 14.26 | 68.92 | |
| 6 | 15.50 | 13.46 | 65.06 | |
| | | | Mean % soluble protein: | 71.92 |
| | | 2% NaCl | | |
| 1 | 19.40 | 17.36 | 83.91 | |
| 2 | 18.14 | 16.10 | 77.82 | |
| 3 | 18.05 | 16.01 | 77.38 | |
| 4 | 26.16 | 26.12 | 126.24 | |
| 5 | 21.02 | 18.98 | 91.74 | |
| 6 | 19.40 | 17.36 | 83.91 | |
| | | | Mean % soluble protein: | 90.16 |
| | | 3% NaCl | | |
| 1 | 29.37 | 27.33 | 132.09 | |
| 2 | 20.50 | 18.46 | 89.22 | |
| 3 | 30.37 | 28.33 | 136.93 | |
| 4 | 27.05 | 25.01 | 120.88 | |
| 5 | 20.12 | 18.08 | 87.39 | |
| 6 | 30.17 | 28.13 | 135.96 | |
| | | | Mean % soluble protein: | 117.08 |
| | | 4% NaCl | | |
| 1 | 23.00 | 20.96 | 101.30 | |
| 2 | 29.92 | 27.88 | 134.75 | |
| 3 | 21.83 | 19.59 | 94.68 | |
| 4 | 22.50 | 20.46 | 98.89 | |
| 5 | 22.70 | 20.66 | 99.86 | |
| 6 | 25.00 | 22.96 | 110.97 | |
| | | | Mean % soluble protein: | 106.74 |
| | | 5% NaCl | | |
| 1 | 18.06 | 16.02 | 77.43 | |
| 2 | 27.61 | 25.57 | 123.59 | |
| 3 | 20.00 | 17.96 | 86.81 | |
| 4 | 26.00 | 25.96 | 125.47 | |
| 5 | 21.25 | 19.21 | 92.85 | |
| 6 | 20.10 | 18.06 | 87.29 | |
| | | | Mean % soluble protein: | 98.90 |

Absorbance Readings and Percent Soluble Protein in Soy Protein - Xanthan Gum Solutions where:

pH = 7
Gum = 0.05 %
Protein = Hydrolyzed Soy

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | |
|---|---|---|---|---|
| | | 0% NaCl | | |
| 1 | 17.60 | 17.05 | 69.28 | |
| 2 | 22.47 | 21.92 | 89.07 | |
| 3 | 17.91 | 17.36 | 70.54 | |
| 4 | 20.14 | 19.59 | 79.60 | |
| 5 | 20.47 | 19.92 | 80.94 | |
| 6 | 19.66 | 19.11 | 77.65 | |
| | | | Mean % soluble protein: | 77.85 |
| | | 1% NaCl | | |
| 1 | 17.71 | 17.16 | 69.73 | |
| 2 | 17.77 | 17.22 | 69.97 | |
| 3 | 19.19 | 18.64 | 75.74 | |
| 4 | 18.47 | 17.92 | 72.82 | |
| 5 | 17.90 | 17.35 | 70.50 | |
| 6 | 18.30 | 17.75 | 72.13 | |
| | | | Mean % soluble protein: | 71.81 |
| | | 2% NaCl | | |
| 1 | 18.15 | 17.60 | 71.52 | |
| 2 | 18.17 | 17.62 | 71.60 | |
| 3 | 18.17 | 17.62 | 71.60 | |
| 4 | 18.21 | 17.66 | 71.76 | |
| 5 | 18.86 | 18.31 | 74.40 | |
| 6 | 19.03 | 18.48 | 75.09 | |
| | | | Mean % soluble protein: | 72.66 |
| | | 3% NaCl | | |
| 1 | 15.95 | 15.40 | 62.58 | |
| 2 | 18.00 | 17.45 | 70.91 | |
| 3 | 17.66 | 17.11 | 69.52 | |
| 4 | 17.94 | 17.39 | 70.66 | |
| 5 | 18.15 | 17.60 | 71.52 | |
| 6 | 16.98 | 16.43 | 66.76 | |
| | | | Mean % soluble protein: | 68.66 |
| | | 4% NaCl | | |
| 1 | 17.71 | 17.16 | 69.73 | |
| 2 | 17.98 | 17.43 | 70.82 | |
| 3 | 17.52 | 16.97 | 68.96 | |
| 4 | 17.50 | 16.95 | 68.87 | |
| 5 | 18.20 | 17.65 | 71.72 | |
| 6 | 17.60 | 17.05 | 69.28 | |
| | | | Mean % soluble protein: | 69.90 |
| | | 5% NaCl | | |
| 1 | 17.71 | 17.16 | 69.73 | |
| 2 | 17.50 | 16.95 | 68.87 | |
| 3 | 17.77 | 17.22 | 69.97 | |
| 4 | 17.95 | 17.40 | 70.70 | |
| 5 | 19.50 | 18.95 | 77.00 | |
| 6 | 18.79 | 18.24 | 74.12 | |
| | | | Mean % soluble protein: | 71.73 |

Absorbance Readings and Percent Soluble Protein in Soy Protein - Xanthan Gum Solutions where:

pH = 7
Gum = 0.15 %
Protein = Hydrolyzed Soy

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | |
|---|---|---|---|---|
| **0% NaCl** | | | | |
| 1 | 17.93 | 16.75 | 68.06 | 24. |
| 61 | | | | |
| 2 | 19.15 | 17.97 | 73.02 | |
| 3 | 16.30 | 17.12 | 69.57 | |
| 4 | 17.87 | 16.69 | 67.82 | |
| 5 | 17.65 | 16.47 | 66.92 | |
| 6 | 16.63 | 17.45 | 70.91 | |
| | | Mean % soluble protein: | 69.38 | |
| **1% NaCl** | | | | |
| 1 | 12.25 | 11.07 | 44.98 | |
| 2 | 17.77 | 16.59 | 67.41 | |
| 3 | 19.10 | 17.92 | 72.82 | |
| 4 | 16.75 | 17.57 | 71.39 | |
| 5 | 16.66 | 17.50 | 71.11 | |
| 6 | 17.91 | 16.73 | 67.98 | |
| | | Mean % soluble protein: | 65.95 | |
| **2% NaCl** | | | | |
| 1 | 16.16 | 16.98 | 69.00 | |
| 2 | 16.80 | 17.62 | 71.60 | |
| 3 | 17.54 | 16.36 | 66.48 | |
| 4 | 17.62 | 16.44 | 66.80 | |
| 5 | 16.50 | 17.32 | 70.38 | |
| 6 | 16.11 | 16.93 | 68.79 | |
| | | Mean % soluble protein: | 68.84 | |
| **3% NaCl** | | | | |
| 1 | 17.66 | 16.48 | 66.96 | |
| 2 | 19.81 | 18.63 | 75.70 | |
| 3 | 20.00 | 18.82 | 76.47 | |
| 4 | 20.31 | 19.13 | 77.73 | |
| 5 | 19.65 | 18.67 | 75.86 | |
| 6 | 18.23 | 17.05 | 69.28 | |
| | | Mean % soluble protein: | 73.67 | |
| **4% NaCl** | | | | |
| 1 | 18.59 | 17.41 | 70.74 | |
| 2 | 18.10 | 16.92 | 68.75 | |
| 3 | 17.98 | 16.80 | 68.26 | |
| 4 | 18.13 | 16.95 | 68.87 | |
| 5 | 18.45 | 17.27 | 70.17 | |
| 6 | 19.70 | 18.52 | 75.25 | |
| | | Mean % soluble protein: | 70.34 | |
| **5% NaCl** | | | | |
| 1 | 18.90 | 17.72 | 72.00 | |
| 2 | 19.15 | 17.97 | 73.02 | |
| 3 | 15.60 | 14.42 | 58.59 | |
| 4 | 19.50 | 18.32 | 74.44 | |
| 5 | 19.77 | 18.59 | 75.54 | |
| 6 | 18.97 | 17.79 | 72.29 | |
| | | Mean % soluble protein: | 70.98 | |

Absorbance Readings and Percent Soluble Protein in Soy Protein - Xanthan Gum Solutions where:

pH = 7
Gum = 0.25 %
Protein = Hydrolyzed Soy

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein |
|---|---|---|---|
| | | 0% NaCl | |
| 1 | 25.85 | 23.61 | 96.75 |
| 2 | 25.41 | 24.23 | 98.46 |
| 3 | 17.77 | 16.59 | 67.41 |
| 4 | 24.98 | 23.60 | 96.71 |
| 5 | 24.90 | 23.72 | 96.38 |
| 6 | 25.50 | 24.32 | 98.82 |
| | | Mean % soluble protein: | 92.42 |
| | | 1% NaCl | |
| 1 | 23.21 | 22.03 | 89.52 |
| 2 | 25.10 | 23.92 | 97.20 |
| 3 | 25.68 | 24.50 | 99.55 |
| 4 | 23.10 | 21.92 | 89.07 |
| 5 | 24.91 | 23.73 | 96.42 |
| 6 | 25.13 | 23.95 | 97.32 |
| | | Mean % soluble protein: | 94.85 |
| | | 2% NaCl | |
| 1 | 25.30 | 24.12 | 98.01 |
| 2 | 24.63 | 23.45 | 95.29 |
| 3 | 24.31 | 23.13 | 93.99 |
| 4 | 24.32 | 23.14 | 94.03 |
| 5 | 25.87 | 24.69 | 100.33 |
| 6 | 24.90 | 23.72 | 96.38 |
| | | Mean % soluble protein: | 96.34 |
| | | 3% NaCl | |
| 1 | 27.05 | 25.87 | 105.12 |
| 2 | 36.00 | 34.82 | 141.49 |
| 3 | 24.97 | 23.79 | 96.67 |
| 4 | 25.11 | 23.93 | 97.24 |
| 5 | 25.79 | 24.61 | 100.00 |
| 6 | 26.86 | 25.70 | 104.43 |
| | | Mean % soluble protein: | 107.49 |
| | | 4% NaCl | |
| 1 | 27.50 | 26.32 | 106.95 |
| 2 | 21.34 | 20.16 | 81.92 |
| 3 | 25.05 | 23.87 | 96.99 |
| 4 | 24.66 | 23.48 | 95.41 |
| 5 | 24.90 | 23.72 | 96.38 |
| 6 | 23.61 | 22.43 | 91.14 |
| | | Mean % soluble protein: | 94.80 |
| | | 5% NaCl | |
| 1 | 21.12 | 19.94 | 81.02 |
| 2 | 19.64 | 18.46 | 75.01 |
| 3 | 26.78 | 25.60 | 104.02 |
| 4 | 24.36 | 23.18 | 94.19 |
| 5 | 25.97 | 24.79 | 100.73 |
| 6 | 23.32 | 22.14 | 89.96 |
| | | Mean % soluble protein: | 90.82 |

Absorbance Readings and Percent Soluble Protein in Soy Protein-Xanthan Gum Solutions where:

pH = 5
Gum = 0.05%
Protein = Intact Soy

| Sample # | Absorbance | Abs.-Blank | % soluble protein | |
|---|---|---|---|---|
| | | 0% NaCl | | |
| 1 | 9.50 | 8.95 | 62.59 | |
| 2 | 8.26 | 7.71 | 53.92 | |
| 3 | 6.46 | 5.91 | 41.33 | |
| 4 | 5.04 | 4.49 | 31.40 | |
| 5 | 6.50 | 5.95 | 41.61 | |
| 6 | 5.98 | 5.43 | 37.97 | |
| | | | Mean % soluble protein: | 44.80 |
| | | 1% NaCl | | |
| 1 | 5.54 | 4.99 | 34.90 | |
| 2 | 5.50 | 4.95 | 34.62 | |
| 3 | 5.81 | 5.26 | 36.78 | |
| 4 | 5.37 | 4.82 | 33.71 | |
| 5 | 6.75 | 6.20 | 43.36 | |
| 6 | 10.52 | 9.97 | 69.72 | |
| | | | Mean % soluble protein: | 42.18 |
| | | 2% NaCl | | |
| 1 | 8.04 | 7.49 | 52.38 | |
| 2 | 6.68 | 6.13 | 42.87 | |
| 3 | 7.92 | 7.37 | 51:54 | |
| 4 | 6.93 | 6.38 | 44.62 | |
| 5 | 7.56 | 7.01 | 49.02 | |
| 6 | 7.72 | 7.17 | 50.14 | |
| | | | Mean % soluble protein: | 48.43 |
| | | 3% NaCl | | |
| 1 | 8.57 | 8.02 | 56.08 | |
| 2 | 9.24 | 8.69 | 60.77 | |
| 3 | 8.78 | 8.23 | 57.55 | |
| 4 | 9.14 | 8.59 | 60.07 | |
| 5 | 7.40 | 6.85 | 47.90 | |
| 6 | 7.58 | 7.03 | 49.16 | |
| | | | Mean % soluble protein: | 55.26 |
| | | 4% NaCl | | |
| 1 | 14.22 | 13.67 | 95.59 | |
| 2 | 9.13 | 8.58 | 60.00 | |
| 3 | 10.62 | 10.07 | 70.42 | |
| 4 | 8.91 | 8.36 | 58.46 | |
| 5 | 9.17 | 8.62 | 60.28 | |
| 6 | 9.52 | 8.97 | 62.73 | |
| | | | Mean % soluble protein: | 67.91 |
| | | 5% NaCl | | |
| 1 | 13.06 | 12.51 | 87.48 | |
| 2 | 9.11 | 8.56 | 59.86 | |
| 3 | 8.44 | 7.89 | 55.17 | |
| 4 | 9.02 | 8.47 | 59.23 | |
| 5 | 9.10 | 8.55 | 59.79 | |
| 6 | 9.37 | 8.82 | 61.68 | |
| | | | Mean % soluble protein: | 63.87 |

Absorbance Readings and Percent Soluble Protein in Soy Protein-Xanthan Gum Solutions where:

pH = 5
Gum = 0.15 %
Protein = Intact Soy

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein |
|---|---|---|---|
| | | 0% NaCl | |
| 1 | 11.03 | 9.85 | 68.88 |
| 2 | 4.91 | 3.73 | 26.08 |
| 3 | 7.59 | 6.41 | 44.83 |
| 4 | 6.60 | 5.42 | 37.90 |
| 5 | 10.74 | 9.56 | 66.85 |
| 6 | 12.36 | 11.18 | 78.18 |
| | | Mean % soluble protein: | 53.79 |
| | | 1% NaCl | |
| 1 | 10.00 | 8.82 | 61.68 |
| 2 | 12.93 | 11.75 | 82.17 |
| 3 | 9.79 | 8.61 | 60.21 |
| 4 | 19.15 | 17.97 | 125.66 |
| 5 | 8.65 | 7.47 | 52.24 |
| 6 | 11.50 | 10.32 | 72.17 |
| | | Mean % soluble protein: | 75.69 |
| | | 2% NaCl | |
| 1 | 11.94 | 10.76 | 75.24 |
| 2 | 10.23 | 9.05 | 63.29 |
| 3 | 11.00 | 9.82 | 68.67 |
| 4 | 9.43 | 8.25 | 57.69 |
| 5 | 5.71 | 4.53 | 31.68 |
| 6 | 12.52 | 11.34 | 79.30 |
| | | Mean % soluble protein: | 62.65 |
| | | 3% NaCl | |
| 1 | 15.49 | 14.31 | 100.07 |
| 2 | 17.95 | 16.77 | 117.27 |
| 3 | 10.10 | 8.92 | 62.38 |
| 4 | 17.62 | 16.44 | 114.97 |
| 5 | 9.74 | 8.56 | 59.86 |
| 6 | 10.26 | 9.08 | 63.50 |
| | | Mean % soluble protein: | 86.34 |
| | | 4% NaCl | |
| 1 | 23.00 | 21.82 | 152.59 |
| 2 | 12.90 | 11.72 | 81.96 |
| 3 | 10.62 | 9.44 | 66.01 |
| 4 | 12.23 | 11.05 | 77.27 |
| 5 | 13.20 | 12.02 | 84.06 |
| 6 | 11.59 | 10.41 | 72.80 |
| | | Mean % soluble protein: | 89.11 |
| | | 5% NaCl | |
| 1 | 10.73 | 9.55 | 66.78 |
| 2 | 12.25 | 11.07 | 77.41 |
| 3 | 12.10 | 10.92 | 76.36 |
| 4 | 24.02 | 22.84 | 159.72 |
| 5 | 13.64 | 12.46 | 87.13 |
| 6 | 12.60 | 11.42 | 79.86 |
| | | Mean % soluble protein: | 91.21 |

Absorbance Readings and Percent Soluble Protein in Soy Protein - Xanthan Gum Solutions where:

pH = 5
Gum = 0.25 %
Protein = Intact Soy

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein |
|---|---|---|---|
| **0% NaCl** | | | |
| 1 | 6.38 | 4.34 | 30.35 |
| 2 | 9.26 | 8.08 | 56.50 |
| 3 | 7.48 | 6.30 | 44.02 |
| 4 | 12.30 | 11.12 | 77.76 |
| 5 | 11.52 | 10.34 | 72.31 |
| 6 | 8.76 | 7.58 | 53.01 |
| | | Mean % soluble protein: | 55.66 |
| **1% NaCl** | | | |
| 1 | 8.88 | 7.70 | 53.81 |
| 2 | 12.86 | 11.68 | 81.68 |
| 3 | 9.57 | 8.39 | 58.67 |
| 4 | 12.87 | 11.69 | 81.75 |
| 5 | 13.26 | 12.08 | 84.44 |
| 6 | 12.87 | 11.69 | 81.75 |
| | | Mean % soluble protein: | 73.68 |
| **2% NaCl** | | | |
| 1 | 12.50 | 11.32 | 79.16 |
| 2 | 12.80 | 11.62 | 81.22 |
| 3 | 11.72 | 10.54 | 73.71 |
| 4 | 17.55 | 16.37 | 114.48 |
| 5 | 26.14 | 24.96 | 174.51 |
| 6 | 12.65 | 11.47 | 80.21 |
| | | Mean % soluble protein: | 100.55 |
| **3% NaCl** | | | |
| 1 | 14.78 | 13.60 | 95.10 |
| 2 | 13.52 | 12.34 | 86.29 |
| 3 | 13.29 | 12.11 | 84.69 |
| 4 | 14.80 | 13.62 | 95.21 |
| 5 | 12.87 | 11.69 | 81.71 |
| 6 | 13.26 | 12.08 | 84.48 |
| | | Mean % soluble protein: | 87.91 |
| **4% NaCl** | | | |
| 1 | 11.50 | 10.32 | 72.17 |
| 2 | 14.44 | 13.26 | 92.73 |
| 3 | 15.23 | 14.05 | 98.22 |
| 4 | 15.75 | 14.57 | 101.89 |
| 5 | 12.21 | 11.03 | 77.13 |
| 6 | 13.81 | 12.63 | 88.29 |
| | | Mean % soluble protein: | 88.40 |
| **5% NaCl** | | | |
| 1 | 16.38 | 15.20 | 106.29 |
| 2 | 14.91 | 13.73 | 96.01 |
| 3 | 10.20 | 9.02 | 63.08 |
| 4 | 15.12 | 13.94 | 97.48 |
| 5 | 8.30 | 7.12 | 49.76 |
| 6 | 13.10 | 11.92 | 83.36 |
| | | Mean % soluble protein: | 82.66 |

Absorbance Readings and Percent Soluble Protein in Soy Protein-Xanthan Gum Solutions where:

pH = 5
Gum = 0.05%
Protein = Hydrolyzed Soy

| Sample # | Absorbance | Abs.-Blank | % Soluble Protein | |
|---|---|---|---|---|
| | | 0% NaCl | | |
| 1 | 18.15 | 17.60 | 71.03 | |
| 2 | 17.05 | 16.50 | 66.59 | |
| 3 | 18.46 | 17.93 | 72.36 | |
| 4 | 18.04 | 17.49 | 70.58 | |
| 5 | 18.26 | 17.71 | 71.47 | |
| 6 | 17.16 | 16.61 | 67.03 | |
| | | | Mean % soluble protein: | 69.84 |
| | | 1% NaCl | | |
| 1 | 18.54 | 17.99 | 72.60 | |
| 2 | 15.26 | 14.71 | 59.36 | |
| 3 | 17.26 | 16.71 | 67.43 | |
| 4 | 18.25 | 17.70 | 71.43 | |
| 5 | 17.97 | 17.42 | 70.30 | |
| 6 | 16.65 | 16.10 | 64.97 | |
| | | | Mean % soluble protein: | 67.68 |
| | | 2% NaCl | | |
| 1 | 19.36 | 18.81 | 75.91 | |
| 2 | 18.56 | 18.01 | 72.68 | |
| 3 | 16.12 | 15.57 | 62.83 | |
| 4 | 17.44 | 16.89 | 68.16 | |
| 5 | 17.98 | 17.43 | 70.34 | |
| 6 | 18.37 | 17.82 | 71.91 | |
| | | | Mean % soluble protein: | 70.31 |
| | | 3% NaCl | | |
| 1 | 12.49 | 11.94 | 48.18 | |
| 2 | 18.92 | 18.37 | 74.13 | |
| 3 | 16.50 | 15.95 | 64.37 | |
| 4 | 17.22 | 16.67 | 67.27 | |
| 5 | 16.43 | 15.88 | 64.08 | |
| 6 | 16.35 | 15.80 | 63.76 | |
| | | | Mean % soluble protein: | 63.63 |
| | | 4% NaCl | | |
| 1 | 18.15 | 17.60 | 71.03 | |
| 2 | 18.26 | 17.71 | 71.47 | |
| 3 | 16.80 | 16.25 | 65.58 | |
| 4 | 17.95 | 17.40 | 70.22 | |
| 5 | 17.27 | 16.72 | 67.47 | |
| 6 | 17.86 | 17.31 | 69.85 | |
| | | | Mean % soluble protein: | 69.27 |
| | | 5% NaCl | | |
| 1 | 17.86 | 17.31 | 69.85 | |
| 2 | 17.86 | 17.31 | 69.85 | |
| 3 | 17.86 | 17.31 | 69.85 | |
| 4 | 17.57 | 17.02 | 68.68 | |
| 5 | 17.23 | 16.68 | 67.31 | |
| 6 | 17.93 | 17.38 | 70.14 | |
| | | | Mean % soluble protein: | 69.28 |

Absorbance Readings and Percent Soluble Protein in Soy Protein-Xanthan Gum Solutions where:

pH = 5
Gum = 0.15%
Protein = Hydrolyzed Soy

| Sample # | Absorbance | Abs.-Blank | % Soluble Protein | DSPI |
|---|---|---|---|---|
| | | 0% NaCl | | |
| 1 | 18.68 | 17.50 | 70.62 | 24.78 |
| 2 | 18.90 | 17.72 | 71.51 | 24.78 |
| 3 | 18.19 | 17.01 | 65.83 | 25.84 |
| 4 | 19.14 | 17.96 | 69.50 | 25.84 |
| 5 | 15.71 | 14.53 | 58.64 | 24.78 |
| 6 | 18.22 | 17.04 | 68.77 | 24.78 |
| | Mean % soluble protein: | | 67.48 | |
| | | 1% NaCl | | |
| 1 | 20.08 | 18.90 | 73.14 | 25.84 |
| 2 | 17.46 | 16.28 | 63.00 | 25.84 |
| 3 | 21.00 | 19.82 | 79.98 | 24.78 |
| 4 | 20.99 | 19.81 | 79.94 | 24.78 |
| 5 | 19.14 | 17.96 | 72.48 | 24.78 |
| 6 | 19.89 | 18.71 | 75.50 | 24.78 |
| | Mean % soluble protein: | | 74.01 | |
| | | 2% NaCl | | |
| 1 | 19.91 | 18.73 | 72.48 | 25.84 |
| 2 | 17.50 | 16.32 | 63.16 | 25.84 |
| 3 | 18.20 | 17.02 | 65.87 | 25.84 |
| 4 | 19.25 | 18.07 | 72.92 | 24.78 |
| 5 | 18.13 | 16.95 | 68.40 | 24.78 |
| 6 | 18.73 | 17.55 | 70.82 | 24.78 |
| | Mean % soluble protein: | | 68.94 | |
| | | 3% NaCl | | |
| 1 | 18.37 | 17.19 | 69.37 | 24.78 |
| 2 | 19.03 | 17.85 | 72.03 | 24.78 |
| 3 | 23.32 | 22.14 | 89.35 | 24.78 |
| 4 | 19.97 | 18.79 | 75.83 | 24.78 |
| 5 | 20.13 | 18.95 | 76.47 | 24.78 |
| 6 | 19.75 | 18.57 | 74.94 | 24.78 |
| | Mean % soluble protein: | | 76.33 | |
| | | 4% NaCl | | |
| 1 | 18.59 | 17.41 | 67.38 | 25.84 |
| 2 | 21.23 | 20.05 | 77.59 | 25.84 |
| 3 | 19.60 | 18.62 | 75.14 | 24.78 |
| 4 | 19.97 | 18.79 | 75.83 | 24.78 |
| 5 | 18.98 | 17.80 | 71.83 | 24.78 |
| 6 | 18.69 | 17.51 | 67.76 | 25.84 |
| | Mean % soluble protein: | | 72.59 | |
| | | 5% NaCl | | |
| 1 | 19.03 | 17.85 | 69.08 | 25.84 |
| 2 | 19.24 | 18.06 | 69.89 | 25.84 |
| 3 | 19.17 | 17.99 | 72.60 | 24.78 |
| 4 | 18.98 | 17.80 | 71.83 | 24.78 |
| 5 | 18.89 | 17.71 | 71.47 | 24.78 |
| 6 | 19.22 | 18.04 | 72.80 | 24.78 |
| | Mean % soluble protein: | | 71.28 | |

Absorbance Readings and Percent Soluble Protein in Soy Protein-Xanthan Gum Solutions where:

pH = 5
Gum = 0.25%
Protein = Hydrolyzed Soy

| Sample # | Absorbance | Abs.-Blank | % Soluble Protein | DSPI |
|---|---|---|---|---|
| | | 0% NaCl | | |
| 1 | 29.82 | 27.78 | 112.11 | 24.78 |
| 2 | 22.10 | 20.06 | 77.63 | 25.84 |
| 3 | 27.86 | 25.82 | 99.92 | 25.84 |
| 4 | 18.26 | 16.22 | 65.46 | 24.78 |
| 5 | 27.79 | 25.75 | 99.65 | 25.84 |
| 6 | 27.50 | 25.46 | 102.74 | 24.78 |
| | Mean % soluble protein: | | 92.92 | |
| | | 1% NaCl | | |
| 1 | 18.39 | 16.35 | 65.98 | 24.78 |
| 2 | 20.76 | 18.72 | 75.54 | 24.78 |
| 3 | 25.11 | 23.07 | 93.10 | 24.78 |
| 4 | 27.07 | 25.03 | 101.01 | 24.78 |
| 5 | 18.36 | 16.32 | 65.86 | 24.78 |
| 6 | 18.57 | 16.53 | 66.71 | 24.78 |
| | Mean % soluble protein: | | 78.03 | |
| | | 2% NaCl | | |
| 1 | 24.09 | 22.05 | 88.98 | 24.78 |
| 2 | 27.28 | 25.24 | 101.86 | 24.78 |
| 3 | 34.65 | 32.61 | 131.60 | 24.78 |
| 4 | 29.95 | 27.91 | 112.63 | 24.78 |
| 5 | 24.53 | 22.49 | 90.76 | 24.78 |
| 6 | 24.69 | 22.65 | 91.40 | 24.78 |
| | Mean % soluble protein: | | 102.87 | |
| | | 3% NaCl | | |
| 1 | 36.12 | 34.08 | 137.53 | 24.78 |
| 2 | 29.69 | 27.65 | 111.58 | 24.78 |
| 3 | 37.12 | 35.08 | 141.57 | 24.78 |
| 4 | 33.74 | 31.70 | 127.93 | 24.78 |
| 5 | 34.79 | 32.75 | 132.16 | 24.78 |
| 6 | 28.95 | 26.91 | 108.60 | 24.78 |
| | Mean % soluble protein: | | 126.56 | |
| | | 4% NaCl | | |
| 1 | 23.10 | 21.06 | 84.99 | 24.78 |
| 2 | 37.25 | 35.21 | 142.09 | 24.78 |
| 3 | 31.12 | 29.08 | 117.35 | 24.78 |
| 4 | 36.72 | 34.68 | 139.95 | 24.78 |
| 5 | 23.54 | 21.50 | 86.76 | 24.78 |
| 6 | 21.64 | 19.60 | 79.10 | 24.78 |
| | Mean % soluble protein: | | 108.37 | |
| | | 5% NaCl | | |
| 1 | 26.84 | 24.80 | 100.08 | 24.78 |
| 2 | 28.60 | 26.56 | 107.18 | 24.78 |
| 3 | 25.27 | 23.23 | 93.74 | 24.78 |
| 4 | 26.69 | 24.65 | 99.48 | 24.78 |
| 5 | 24.98 | 22.94 | 92.57 | 24.78 |
| 6 | 26.10 | 24.06 | 97.09 | 24.78 |
| | Mean % soluble protein: | | 98.36 | |

Absorbance Readings and Percent Soluble Protein in Whey Protein-Xanthan Gum Solutions where:

pH = 7
Gum = 0.25%
Protein = Intact Whey

| Sample # | Absorbance | Abs.-Blank | % Soluble Protein | Whey |
|---|---|---|---|---|
| | | 1% NaCl | | |
| 1 | 0.918 | 0.714 | 62.963 | 1.134 |
| 2 | 0.971 | 0.767 | 67.637 | 1.134 |
| 3 | 0.922 | 0.718 | 63.316 | 1.134 |
| 4 | 0.970 | 0.766 | 67.549 | 1.134 |
| 5 | 0.960 | 0.756 | 66.667 | 1.134 |
| 6 | 0.847 | 0.643 | 56.702 | 1.134 |
| | Mean % soluble protein: | | 64.139 | |
| | | 2% NaCl | | |
| 1 | 0.896 | 0.692 | 61.023 | 1.134 |
| 2 | 0.989 | 0.785 | 69.224 | 1.134 |
| 3 | 0.901 | 0.697 | 61.464 | 1.134 |
| 4 | 0.899 | 0.695 | 61.287 | 1.134 |
| | Mean % soluble protein: | | 63.250 | |
| | | 3% NaCl | | |
| 1 | 0.903 | 0.699 | 61.640 | 1.134 |
| 2 | 0.948 | 0.744 | 65.608 | 1.134 |
| 3 | 0.950 | 0.746 | 65.785 | 1.134 |
| 4 | 0.972 | 0.768 | 67.725 | 1.134 |
| 5 | 0.967 | 0.763 | 67.284 | 1.134 |
| 6 | 0.941 | 0.737 | 64.991 | 1.134 |
| | Mean % soluble protein: | | 65.506 | |
| | | 4% NaCl | | |
| 1 | 0.964 | 0.760 | 67.019 | 1.134 |
| 2 | 0.968 | 0.764 | 67.372 | 1.134 |
| 3 | 0.947 | 0.743 | 65.520 | 1.134 |
| 4 | 0.952 | 0.748 | 65.961 | 1.134 |
| 5 | 0.878 | 0.674 | 59.436 | 1.134 |
| 6 | 0.996 | 0.792 | 69.841 | 1.134 |
| | Mean % soluble protein: | | 65.858 | |
| | | 5% NaCl | | |
| 1 | 0.892 | 0.688 | 60.670 | 1.134 |
| 2 | 0.831 | 0.627 | 55.291 | 1.134 |
| 3 | 0.887 | 0.683 | 60.229 | 1.134 |
| 4 | 0.903 | 0.699 | 61.640 | 1.134 |
| 5 | 0.981 | 0.777 | 68.519 | 1.134 |
| 6 | 0.861 | 0.657 | 57.937 | 1.134 |
| | Mean % soluble protein: | | 60.714 | |

Absorbance Readings and Percent Soluble Protein in Whey Protein-Xanthan Gum Solutions where:

pH = 5
Gum = 0.15%
Protein = Intact Whey

| Sample # | Absorbance | Abs.-Blank | % Soluble Protein | Whey |
|---|---|---|---|---|
| | | 1% NaCl | | |
| 1.000 | 0.763 | 0.645 | 56.878 | 1.134 |
| 2.000 | 0.769 | 0.651 | 57.407 | 1.134 |
| 3.000 | 0.973 | 0.855 | 75.397 | 1.134 |
| 4.000 | 1.029 | 0.911 | 80.335 | 1.134 |
| 5.000 | 0.836 | 0.718 | 63.316 | 1.134 |
| 6.000 | 0.839 | 0.721 | 63.580 | 1.134 |
| | Mean % soluble protein: | | 66.152 | |
| | | 2% NaCl | | |
| 1 | 0.782 | 0.664 | 58.554 | 1.134 |
| 2 | 0.817 | 0.699 | 61.640 | 1.134 |
| 3 | 0.759 | 0.641 | 56.526 | 1.134 |
| 4 | 0.806 | 0.688 | 60.670 | 1.134 |
| 5 | 0.761 | 0.663 | 58.466 | 1.134 |
| 6 | 1.052 | 0.934 | 82.363 | 1.134 |
| | Mean % soluble protein: | | 63.036 | |
| | | 3% NaCl | | |
| 1 | 0.855 | 0.651 | 57.407 | 1.134 |
| 2 | 0.926 | 0.722 | 63.668 | 1.134 |
| 3 | 0.804 | 0.600 | 52.910 | 1.134 |
| 4 | 0.799 | 0.595 | 52.469 | 1.134 |
| 5 | 0.865 | 0.661 | 58.289 | 1.134 |
| 6 | 1.097 | 0.893 | 78.748 | 1.134 |
| | Mean % soluble protein: | | 60.582 | |
| | | 4% NaCl | | |
| 1 | 0.878 | 0.674 | 59.436 | 1.134 |
| 2 | 0.912 | 0.708 | 62.434 | 1.134 |
| 3 | 0.873 | 0.669 | 58.995 | 1.134 |
| 4 | 0.890 | 0.686 | 60.494 | 1.134 |
| 5 | 1.147 | 0.943 | 83.157 | 1.134 |
| 6 | 0.999 | 0.795 | 70.106 | 1.134 |
| | Mean % soluble protein: | | 65.770 | |

Absorbance Readings and Percent Soluble Protein in Whey Protein-Xanthan Gum Solutions where:

pH = 5
Gum = 0.25%
Protein = Intact Whey

| Sample # | Absorbance | Abs.-Blank | % Soluble Protein | Whey |
|---|---|---|---|---|
| | | 2% NaCl | | |
| 1 | 1.047 | 0.843 | 74.339 | 1.134 |
| 2 | 1.059 | 0.855 | 75.397 | 1.134 |
| 3 | 0.971 | 0.767 | 67.637 | 1.134 |
| 4 | 0.892 | 0.688 | 60.670 | 1.134 |
| 5 | 1.154 | 0.950 | 83.774 | 1.134 |
| 6 | 1.998 | 1.794 | 158.201 | 1.134 |
| | Mean % soluble protein: | | 86.670 | |
| | | 3% NaCl | | |
| 1 | 1.072 | 0.868 | 76.543 | 1.134 |
| 2 | 1.063 | 0.859 | 75.750 | 1.134 |
| 3 | 0.999 | 0.795 | 70.106 | 1.134 |
| 4 | 1.134 | 0.930 | 82.011 | 1.134 |
| 5 | 1.127 | 0.923 | 81.393 | 1.134 |
| 6 | 1.056 | 0.852 | 75.132 | 1.134 |
| | Mean % soluble protein: | | 76.822 | |
| Sample # | Absorbance | Abs.-Blank | % Soluble Protein | Whey |
| | | 4% NaCl | | |
| 1 | 1.187 | 0.983 | 86.684 | 1.134 |
| 2 | 1.179 | 0.975 | 85.979 | 1.134 |
| 3 | 1.240 | 1.036 | 91.358 | 1.134 |
| 4 | 1.285 | 1.081 | 95.326 | 1.134 |
| 5 | 1.175 | 0.971 | 85.626 | 1.134 |
| 6 | 1.240 | 1.036 | 91.358 | 1.134 |
| | Mean % soluble protein: | | 89.389 | |
| | | 5% NaCl | | |
| 1 | 1.215 | 1.011 | 89.153 | 1.134 |
| 2 | 1.317 | 1.113 | 98.146 | 1.134 |
| 3 | 1.275 | 1.071 | 94.444 | 1.134 |
| 4 | 1.331 | 1.127 | 99.383 | 1.134 |
| 5 | 1.132 | 0.928 | 81.834 | 1.134 |
| 6 | 1.171 | 0.967 | 85.273 | 1.134 |
| | Mean % soluble protein: | | 91.373 | |

Absorbance Readings and Percent Soluble Protein in Soy Protein - Xanthan Gum Solutions where:

pH = 2
Gum = 0.25 %
Protein = Intact Soy
NaCl = 3%

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | Control |
|---|---|---|---|---|
| 1 | 20.16 | 18.12 | 101.29 | 17.89 |
| 2 | 3.09 | 1.05 | 5.87 | 17.89 |
| 3 | 1.20 | -0.84 | -4.70 | 17.89 |
| 4 | 11.68 | 9.64 | 53.88 | 17.89 |
| 5 | 5.28 | 3.24 | 18.11 | 17.89 |
| 6 | 3.79 | 1.75 | 9.78 | 17.89 |

Mean % soluble protein:30.71

pH = 3
Gum = 0.25 %
Protein = Intact Soy
NaCl = 3%

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | Control |
|---|---|---|---|---|
| 1 | 13.36 | 11.32 | 58.47 | 19.36 |
| 2 | 13.25 | 11.21 | 57.90 | 19.36 |
| 3 | 15.62 | 13.58 | 70.14 | 19.36 |
| 4 | 14.70 | 12.66 | 65.39 | 19.36 |
| 5 | 14.94 | 12.90 | 66.63 | 19.36 |
| 6 | 15.33 | 13.29 | 68.65 | 19.36 |

Mean % soluble protein: 64.53

pH = 4
Gum = 0.25 %
Protein = Intact Soy
NaCl = 3%

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | Control |
|---|---|---|---|---|
| 1 | 17.63 | 15.59 | 83.86 | 18.59 |
| 2 | 20.48 | 18.44 | 99.19 | 18.59 |
| 3 | 22.53 | 20.49 | 110.22 | 18.59 |
| 4 | 22.05 | 20.01 | 107.64 | 18.59 |
| 5 | 20.50 | 18.46 | 101.37 | 18.21 |
| 6 | 20.25 | 18.21 | 100.00 | 18.21 |

Mean % soluble protein:100.38

pH = 9
Gum = 0.25 %
Protein = Intact Soy
NaCl = 3%

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | Control |
|---|---|---|---|---|
| 1 | 26.23 | 24.19 | 87.58 | 27.62 |
| 2 | 20.73 | 18.69 | 67.67 | 27.62 |
| 3 | 20.54 | 18.50 | 66.98 | 27.62 |
| 4 | 24.70 | 22.66 | 82.04 | 27.62 |
| 5 | 21.42 | 19.38 | 70.17 | 27.62 |
| 6 | 21.48 | 19.44 | 70.38 | 27.62 |

Mean % soluble protein: 74.14

Absorbance Readings and Percent Soluble Protein in Soy Protein - Xanthan Gum Solutions where:

pH = 2
Gum = 0.25 %
Protein = Hydrolyzed Soy
NaCl = 3%

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | Control |
|---|---|---|---|---|
| 1 | 24.21 | 22.17 | 104.43 | 21.23 |
| 2 | 28.18 | 26.14 | 123.13 | 21.23 |
| 3 | 22.17 | 20.13 | 94.82 | 21.23 |
| 4 | 26.14 | 24.10 | 113.52 | 21.23 |
| 5 | 18.21 | 16.17 | 76.17 | 21.23 |
| 6 | 21.12 | 19.08 | 89.87 | 21.23 |

Mean % soluble protein:100.32

pH = 3
Gum = 0.25 %
Protein = Hydrolyzed Soy
NaCl = 3%

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | Control |
|---|---|---|---|---|
| 1 | 20.20 | 18.16 | 95.63 | 18.95 |
| 2 | 18.16 | 16.12 | 85.07 | 18.95 |
| 3 | 21.88 | 19.84 | 104.70 | 18.95 |
| 4 | 19.84 | 17.80 | 93.93 | 18.95 |
| 5 | 18.21 | 16.17 | 87.08 | 18.57 |
| 6 | 18.21 | 16.17 | 87.08 | 18.57 |

Mean % soluble protein: 92.28

pH = 4
Gum = 0.25 %
Protein = Hydrolyzed Soy
NaCl = 3%

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | Control |
|---|---|---|---|---|
| 1 | 30.14 | 28.10 | 113.40 | 24.78 |
| 2 | 32.96 | 30.92 | 124.78 | 24.78 |
| 3 | 28.00 | 25.96 | 104.76 | 24.78 |
| 4 | 30.91 | 28.87 | 116.51 | 24.78 |
| 5 | 30.29 | 28.25 | 114.00 | 24.78 |
| 6 | 29.75 | 27.71 | 111.82 | 24.78 |

Mean % soluble protein:114.21

pH = 9
Gum = 0.25 %
Protein = Hydrolyzed Soy
NaCl = 3%

| Sample # | Absorbance | Abs. - Blank | % Soluble Protein | Control |
|---|---|---|---|---|
| 1 | 23.04 | 21.00 | 97.45 | 21.55 |
| 2 | 23.60 | 21.56 | 100.05 | 21.55 |
| 3 | 21.00 | 18.96 | 87.96 | 21.55 |
| 4 | 21.56 | 19.52 | 90.58 | 21.55 |
| 5 | 22.79 | 20.75 | 96.29 | 21.55 |
| 6 | 22.83 | 20.79 | 98.11 | 21.19 |

Mean % soluble protein: 95.06

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