DISPERSIBILITY INDICES, EMULSION CAPACITIES, AND ELECTROPHORETIC COMPARISONS OF PROTEIN EXTRACTED FROM DEFATTED SOY FLAKE SUSPENSIONS AT pH 4.5, pH 3.0, AND pH 3.0 IN THE PRESENCE OF CALCIUM CHLORIDE

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CHAPTER I

INTRODUCTION

The ability of the food industry to utilize soybean protein for its functional properties had been severely hampered by the lack of soy protein concentrate and isolate solubility. The ability of a soy protein isolate to emulsify, absorb fats, form films, modify texture, and form foams, is adversely affected by the reaction of phytic and acid with certain protein fractions. Although many factors may be responsible for poor functional properties of soy isolates, insoluble complexes formed with phytic acid is a major one.

The removal of phytic acid from soy isolates has been accomplished by two modifications of the commercial procedure used to prepare soy protein isolates. These modifications involve preparing alkaline suspensions of the protein obtained from defatted soy flakes and producing two isolates with low phytic acid content by 1) adjusting the pH of the suspension to 3.0 rather than 4.5 (the average isoelectric point of soy protein) and 2) adjusting the pH to 3.0 in the presence of calcium chloride.

The objectives of this research were:

1. To compare the yields of the two isolates prepared by the modified procedures mentioned above, to an isolate prepared by the commercial method;

2. To determine the differences in the functional properties of a) dispersibility and b) emulsion capacity of the two isolates prepared by the modified procedures and an isolate prepared by the commercial method; and

3. To measure differences of soluble proteins of the two isolates prepared by the modified procedures and an isolate prepared by the standard commercial procedures using polyacrylamide gel electrophoresis techniques.

CHAPTER II

REVIEW OF LITERATURE

Introduction

In the United States today, food grade proteins from oilseeds are being produced at a volume estimated to range between 15 to 38 million pounds a year (Meyer, 1971). The soybean (Glycine Max) produces most of this protein and has a production potential of about 27 billion pounds of protein (Smith and Circle, 1972). The protein of soybean has a high lysine content which makes it useful in supplementing other cereal proteins deficient in this amino acid (Wolfe, 1970).

In order to utilize this excellent source of high quality protein and avoid the beany flavor present in the soybean flake, it is advantageous to remove the protein from the soybean flake to form isolates. Isolates have the advantage over soy flour in that they are almost bland in flavor, are white in color, and exhibit excellent keeping quality (Cogan, et al, 1967).

Although isolates cost as much as three to five times more than soy flour, it is worth the extra expense for use in such foods as sausage-type products, meat

analogs, and as an additive in protein fortified beverages (Smith and Wolfe, 1961; Cogan, et al, 1967). Soy protein, when placed in aqueous dispersions, displays many beneficial properties such as moisture absorption, emulsification, whippability, thickening ability, and the capability of forming films, fibers, doughs, and gels (Circle, et al, 1964). These properties are limited by the reaction of phytic acid with the soy proteins. By reducing the solubility of the protein by as much as seventy percent the phytic acid can cause a reduction of functional properties by approximately the same percentage (Bau, et al, 1978; Circle, et al, 1964; Okubo, et al, 1975). Wolf (1970) observed that to obtain desirable functional properties in an isolate, protein solubility was required.

Solubility

Solubility of a soy isolate is the major factor in its potential to develop as a food source (Walker, 1978). A wide variety of processes are used to produce soy protein isolates, such as cold-precipitation, acid-precipitation, salt-precipitation and ultra filtration methods (Nash and Wolfe, 1967; Bau, et al, 1978; Walker, 1978). Each of these methods give isolates which have different solubility characteristics.

Many studies have indicated that solubility of soy isolates increase as pH increases or decreases from the

isoelectric point of soy protein (Smith and Rackis, 1957; Smith and Circle, 1972; van Megen, 1974; Bau, et al, 1978). This is true of isolates even when denatured by the treatment of acids, alkalis, urea, detergents, and heat above 100°C (Wolf, 1970).

One of the major causes of insolubility in soy proteins is denaturation occurring when phytic acid reacts with soy protein (Okubo, et al, 1975). This reaction has caused many researchers to recommend that phytin be removed before attempting to study the solubility of soy isolates or concentrates (Smith and Rackis, 1975; Smith and Circle, 1972). Walker (1978) devised a method to remove phytin and improve the solubility of a soy isolate.

Walker (1978) proposed extraction of phytin from soy isolate by lowering the pH of soy milk solution to 3.0, with subsequent addition of calcium chloride ($CaCl_2$). The calcium-phytin complex was then centrifuged, and protein was then removed by raising the pH of the soy milk to 4.5. This process, according to Walker (1978), yields enough isolate to be commercially feasible.

Soy protein because of its negative charge exhibits increased solubility at high pH. During protein isolation however, the negative charge of the protein attracts calcium ions. The divalent calcium ions then bind the negatively charged phytate molecules to the protein. This complex of protein, calcium, and phytin thus forms a

particle that is very insoluble in aqueous medium (Paul, 1972; Okubo, et al, 1975). This suggests that soy isolate solubility may be improved by removing the phytate (Chang, et al, 1972; Goodnight and Hartman, 1977; Okubo, et al, 1975; Smith and Rackis, 1975).

Dispersibility

Dispersibility is a very important, desirable property in a soy isolate (Circle, et al, 1964; Johnson, 1970; Wu and Inglett, 1974; McWatters and Cherry, 1977; Wang, 1978). Dispersibility is the ability of small solid particles to stay suspended in a liquid medium and not precipitate (Circle, et al, 1964). Methods used to measure dispersibility are empirical and difficult to duplicate (Johnson, 1970). Also, the properties of laboratory-prepared isolates may vary depending on method and control of manufacture (Circle, et al, 1964).

It has been found that emulsion capacity of soy isolates is affected by dispersibility, protein concentration and pH of the dispersion (Hutton and Campbell, 1977). It has been demonstrated that soy protein isolates that stay in aqueous dispersion have better emulsification properties (Johnson, 1970; Crenwelge, et al, 1974; Wolf and Cowan, 1975).

There are no absolute, quantitative tests for determining the dispersibility of soy protein isolates

because researchers have not been able to develop a test that gives reproducible results from laboratory to laboratory. Many different methods have been devised to measure dispersibility of soy isolates.

Circle, et al (1964) studied the dispersibility of soy protein by mixing different isolates with water in a blendor and assuming complete dispersion of the isolates. Ehninger and Pratt (1974) attempted to make dispersions of soy isolates and water using an Osterizer blendor. The isolates and water were blended in a pint jar at high speed for two minutes, resulting in complete dispersion. Mattil (1974) also studied the dispersion characteristics of various isolates by making one percent isolate-to-water mixtures, adjusting the pH, holding these mixtures at 37.5°C in a water bath and shaking them for thirty minutes.

Emulsion Capacity

Emulsion capacity was found to have a crude positive correlation with soy protein solubility (Crenwelge, et al, 1974). It also has been found that insoluble proteins of a dispersion are important in the study of emulsion capacity since small insoluble protein particles can collect at oil-water interfaces and prevent coalescence of fat globules (Wolf and Cowan, 1975).

The ability of soy protein isolates to bind

moisture, emulsify fat, and stabilize emulsions is related to its emulsion capacity (Smith and Circle, 1972). Soy proteins are thought to play two roles in emulsification: 1) aid in the formation of oil-in-water emulsions; and 2) stabilize the emulsions once formed (Hutton and Campbell, 1977).

Research has shown that soybean flour exhibits maximum emulsion capacity at pH 6.5 and that an increase in pH to 8.2 does not improve the emulsion capacity or effect it detrimentally. It was also found that by reducing the pH of the soy protein solutions to near their isoelectric point, emulsion capacity was reduced (McWatters and Cherry, 1977). Hutton and Campbell (1977) attributed this to the fact that pH influences the emulsion capacity indirectly by affecting the solubility of the proteins.

Emulsion capacity has proved to be very difficult to standardize and reproduce between laboratories. This difficulty in standardization is caused by the many different methods that researchers have developed to determine the emulsion capacity end point of protein isolates (Marshall, et al, 1975). Standardization has also been hard to accomplish because the precision associated with each method is low (Marshall, et al, 1975). Sudden drops in emulsion viscosity which occur when an emulsion breaks down have been used to determine the emulsion capacity of protein samples (Swift, et al, 1961; Hegarty, et al, 1963;

Pearson, et al, 1965; Inklaar and Fortuin, 1969; McWatters and Cherry, 1975). Swift, et al (1961) states that an experienced operator is needed to attain the required precision with this method.

Variable auto transformers have been used in connection with a microammeter to determine the drop in amperage associated with emulsion breakdown (Smith, et al, 1973; Crenwelge, et al, 1974). This method is limited because it is difficult to detect the true amperage drop which occurs in high or low viscosity samples at the point of emulsion breakdown (Smith, et al, 1973; Crenwelge, et al, 1974).

Electrical resistance was used to determine conductivity change of an emulsion as a current is passed between two electrodes (Webb, et al, 1970). Hag, et al, (1973) found that this method worked well in low viscosity emulsions but not in high viscosity emulsions.

Marshall, et al (1975) developed a method to more accurately determine emulsion end point and, therefore, emulsion capacity by observing increased visibility of oil droplets colored with the biological stain Oil-Red-O at the moment of emulsion collapse. Marshall, et al (1975) discovered that this method worked well except in the case of very dark emulsions.

Electrophoresis

Electrophoretic research on soybean proteins was developed mainly from the growing interest of food manufacturers in the use of soybean protein additives in meat products (Guy, et al, 1973). Much of the electrophoresis work on soybean proteins has involved polyacrylamide or starch electrophoresis techniques (McWatters and Cherry, 1977; Bau, et al, 1978). These studies involved modification of disc electrophoresis techniques developed by Davis (1964). Disc electrophoresis is the technique of concentrating the sample in a very narrow "disc" of gel before actual separation of proteins occurs. The gels used to separate the protein components are disc-shaped and act as a sieve so that separation can be carried out faster and with better results (Pomeranz and Meloan, 1978).

Soy proteins are complex, heterogeneous mixtures of proteins that vary in their amino acid content. Kapoor and Gupta (1977) extracted proteins from soybeans using an acetate buffer (pH 4.8), and separated protein components by the polyacrylanide gel electrophoresis technique of Davis (1964). Proteins that were present were determined by their characteristic mobilities.

Electrophoretic gel protein band patterns differ in many studies depending on the types and varieties of soybeans used and the method of electrophoresis used in each experiment (Guy, et al, 1973; McWatters and Cherry,

1977; Kapoor and Gupta, 1977; Bau, et al, 1978).

In their study of soy proteins McWatters and Cherry (1977) were unable to relate soy protein functionality to protein quality by gel electrophoresis techniques. Upon extraction from the soybean flake, much of the soy protein is denatured. This denatured protein causes separation of the various component proteins to be impaired. Urea, sodium dodecyl sulfate or mercaptoethanol has been used to break down these insoluble, denatured proteins into smaller protein components which are capable of migrating through the gel to give a more effective separation. It has also been demonstrated that increasing the pH of the soy flake suspension to 8.2 improves the solubility of high molecular weight proteins, in seven centimeter long polycrylamide gels (McWatters and Cherry, 1977).

CHAPTER III

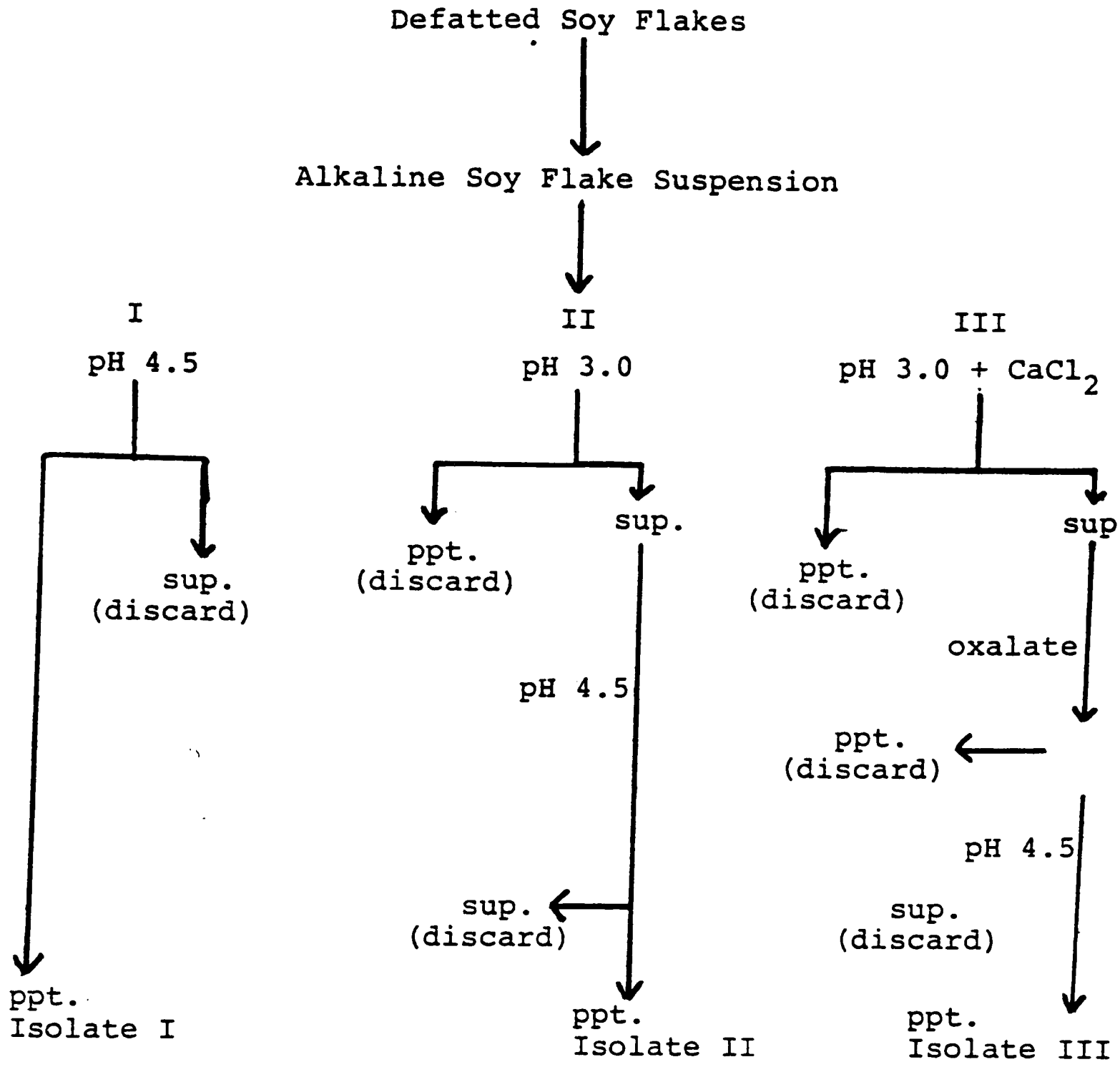
PROCEDURE

Sample Preparation

A flow diagram of the methods used to prepare the pH 4.5 (commercial type), pH 3.0, and pH 3.0 treated with calcium chloride ($CaCl_2$) isolates is shown in Figure 1. Six hundred gram samples of low-heat defatted soy flakes (obtained from the Ralston-Purina Co., St. Louis, Missouri) were slurried with approximately 3300 mls of distilled water in a Waring blendor. The pH of resulting slurry was monitored continuously for one hour and adjusted with 1N NaOH to insure a pH of 8.5-9.0. Slurry was then transferred to one liter centrifuge bottles and centrifuged at 1500 RPM (425 G's) in a VWR, Model GF-8 centrifuge. Supernatant was filtered through glass wool into a clean 16 liter container. This procedure was repeated until container was filled. The container was labeled soy milk.

Aliquots of the soy milk were then treated by Walker's (1978) three methods for isolating soy protein. These methods were:

1. Isolate 1--Adjust pH of 2000 mls of soy milk to 4.5 with IN ltCl in a waring blendor, place solution



sup.-supernatant

ppt.-precipitate

Fig. 1. Flow diagram of sample preparation.

in 1 liter centrifuge bottles and centrifuge at 1500 RPM for 15 minutes. Supernatant was discarded and precipitate was saved.

2. Isolate 2--Adjust pH of 2000 mls of soy milk to 3.0 by addition of 1N HCl in a Waring blendor. Centrifuge as above and recover supernatant. Supernatant placed in Waring blendor and pH was raised to 4.5 by addition of IN NaOH.

3. Isolate 3--Adjust the pH of 2000 ml of soy milk to 3.0 by addition of 1N HCl. 545 ml of 9.5m $CaCl_2$ was added just prior to centrifugation. Centrifuge as above and save the supernatant. 0.5M potassium oxalate was added to the supernatant until the pH reached 3.5, then oxalic acid was added until pH returned to 3.0. This was done until no further precipitation of calcium oxalate was observed. The solution was centrifuged as above. Supernatant was kept, treated with 1N NaOH until pH 4.5 was reached, centrifuged as above. Precipitate was kept.

After isolates were obtained, they were frozen in 1200 ml Lab Con Co Freeze-drier flasks and lypholized using a Lab Con Co Freeze-drier Model 12 until a moisture level of approximately 10 percent was obtained. The isolates were then placed in a one liter, ceramic ball mill jar, containing 20 ceramic balls (1" diameter) and ground at 120 rpm for 90 minutes on a Norton ball mill. The resultant fine powders of the isolated protein samples

were weighed. The percent yield of isolate was based on the weight of the total soy milk solids used to prepare each protein isolate. The equation for percent yeild was:

$$\% \text{ yield of isolate} = \frac{\text{gms of isolated protein obtained} \times 100}{\text{gms of soy milk used} \times \% \text{ total solids of soy milk}}$$

Samples of the raw soy milk obtained from defatted soy flakes without undergoing Walker's (1978) protein isolation procedures were dried and ground in the same manner as isolates 1, 2, and 3.

Nitrogen Dispersibility

The dispersibility of isolates 1, 2, and 3, dried soy milk, and Pro Fam 90 H/S (a commercial isolate obtained from the Grain Processing Corp., Muscatine, Iowa) was determined by the method of Mattil (1974). This method involved adding one part protein to eighty parts water, adjusting the pH to various levels, and adding distilled water to make to one hundred parts water. The mixture was stored at 37.5°C in a water bath for 40 minutes and shaken on a laboratory shaker for 30 minutes. Glass beads were added to improve agitation. The dispersions were then centrifuged at 2000 rpm (571 Gs) for 20 minutes. Supernatant was filtered through Whatman No. 10 filter paper. Percent nitrogen was then determined by micro-Kjeldahl analysis on 2 mls of the supernatant.

The Mattil (1974) method was slightly modified by

placing 8 gms of sample into 150 ml of distilled water in an Erlenmeyer flask. The pH was adjusted by the addition of either 1N HCl or 1N NaOH to each sample. The pH values used in this study were: 9.5, 7.0, 3.0, and 1.5. Three marbles were placed in each flask. The sample was held at 37.5°C for 40 minutes in a circulating water bath, removed, and shaken by Burrel wrist shaker for 30 minutes. Samples were then centrifuged for 20 minutes at 2000 rpm (571 Gs), filtered through Whatman No. 10 filter paper. Two mls of the supernatant was analyzed for percent nitrogen by the micro-Kjeldahl method of AOAC (1975, Appendix A).

Emulsion Capacity

The emulsion capacities (E.C.) of isolates 1, 2, 3, freeze-dried soy milk (SMFD), fresh soy milk (Fresh SM), and Pro Fam 90 H/S were determined by the method of McWatters and Cherry (1975). 0.25g of sample was introduced into a common Mason canning jar. The jar, which had a 3/8 inch hole drilled through the bottom of it, was then placed on an Osterizer blendor. Fifty mls of 0.1 N NaCl solution, which had been adjusted to pH 9.5 by the addition of 0.1N HCl, was introduced by pipette through the 3/8 inch hole in the bottom of the inverted jar. Fifty mls of corn oil was introduced into the jar and the mixture was blended at low speed for 60 seconds. The blendor was then stopped for 15 seconds to prevent heat buildup in the emulsion.

While mixing at low speed, 25 ml of corn oil was added through the hole in the jar at a rate of 0.5 mls per second, with 15 second periods between each addition of 25 mls of oil. Inversion of the emulsion occurred when there was a decrease in emulsion viscosity. The inversion was characterized by the formation of a thin layer of clear oil formed on top of the water and sample layer. The number of milliliters of oil added to the emulsion before the inversion occurred was the milliliters of oil emulsified.

Electrophoresis

Polyacrylamide gel electrophoresis (PAGE) was run on samples of isolates 1, 2, and 3, freeze-dried soy milk, and Pro Fam 90 H/S. The methods of Davis (1964, Appendix B) was used to determine the protein components present in each sample.

Each of the soy isolate and soy milk samples were prepared by weighing 4 gms of sample into a 125 ml Erlenmeyer flask, adding 100 ml of 0.1N THAM-glycine buffer solution (pH 9.2) and then mixed by the procedure for nitrogen dispersibility previously described. Ten mls of the resulting supernatants were then placed on PAGE gels (prepared as in Appendix B). The PAGE gels were then electrophoresed at 3 milliamperes per tube for approximately 3½ hours in a Searle Electrophoretic Apparatus Model 3-1155 (Buchler Instrucments, Fort Lee, New Jersey). The PAGE

gels were then removed and stained by placing the PAGE gels in coomassie blue stain for four hours. The PAGE gels were then destained overnight in 7% acetic acid in a Bio Rad Lab Model 172A Gel Electrophoresis Destainer.

Gel scans of samples were obtained by placing each gel rod into a pyrex gel scanning tube, placing the tube into the gel scanner, and passing the gels under the light source (579 mm) at a speed of 30 centimeters per hour.

Statistic analysis of the emulsion capacity data was achieved by the ranking sums method of Kramer (1963).

CHAPTER IV

RESULTS AND DISCUSSION

Yield of Protein Isolates

The percent yield for each isolated protein is shown in Table 1. It is observed that the yield of isolate 1 is almost twice as high as the yield of isolate 2 and approximately three times the yield of isolate 3. The lowering of isolate yield for isolates 2 and 3, is caused by the loss of phytate-bound protein in the additional processing steps involved in producing isolate 2 and 3. Isolate 3 illustrates this vividly, since the procedure used to make isolate 3 both lowered the pH to 3.0, added calcium ions, causing more of the phytate denatured proteins to precipitate in the earlier stages of protein isolation. Another reason for the yield being low in isolates 2 and 3 is due to the fact that the more soy protein is abused by various isolation techniques, the lower the solubility, thus large amounts of protein was lost in the preliminary steps of preparing isolate 3. In isolate 3 the oxalate ion combines with the calcium ion or with sugar present in the supernatant to give a precipitate of both nitrogenous and non-nitrogenous composition.

TABLE 1

THE YIELD DATA OF ISOLATED PROTEIN OBTAINED FROM ALKALINE SOY FLAKE SUSPENSIONS AFTER ISOLATION AT pH 4.5, pH 3.0, AND pH 3.0 IN THE PRESENCE OF CALCIUM CHLORIDE

Product	Isolate 1	Isolate 2	Isolate 3
Yield (% of total solids of soy flake suspensions)	75.5	44.5	24.2

Percent Protein of Isolates

The amount of protein in the dried isolates, both laboratory and commercial preparations, and the dried soy milk powder is presented in Table 2. These results illustrate that the percent protein for the isolated protein obtained from the Grain Processing Corporation as well as those obtained by procedures 1 and 2 are in the 90-95 percent range. This indicates that the procedures employed in producing the isolates in procedures 1 and 2 worked well, and yielded a high protein content isolate. Procedure 3 did not produce an isolate of as high a protein content as did procedures 1 and 2, indicating that in a pilot plant type study using modifications of Walker's (1978) methods, the protein is not as easily isolated from other components of the soy milk by procedure 3.

TABLE 2

THE PERCENT TOTAL PROTEIN OF ISOLATED PROTEIN SAMPLES PREPARED BY ISOLATION AT pH 4.5, pH 3.0 AND pH 3.0 IN THE PRESENCE OF CALCIUM CHLORIDE

Product	Isolate 1	Isolate 2	Isolate 3
% Protein (Kjeldahl)	94.1	92.4	73.8

Dispersible Nitrogen

Data associated with the nitrogen dispersibility values for freeze-dried soy milk, Pro-Fam 90 H/S, and the three laboratory prepared isolated protein samples is shown in Table 3. Data obtained for the commercial isolate and laboratory isolates 2 and 3 gave values that were similar to results for solubility in the literature (Bau, et al, 1978). The dispersibility values of the samples were low near the isoelectric point, but increased at both higher and lower pH values. The results obtained in the commercial isolate and isolates 2 and 3 indicated that the additional processing used on these isolates affected the dispersibility of the protein adversely, rather than enhancing the dispersibility by the removal of phytin. This was especially evident in the percent suspended nitrogen observed at pH 7.0. The freeze-dried soy milk and isolate 1 showed high nitrogen dispersibility values at all pH levels including pH 7.0, whereas the commercial isolate and

isolates 2 and 3 gave dispersibility values at pH 7.0 that were lower than the other pH levels. This difference can be attributed to the fact that the soy milk and isolate 1 were subjected to much less abuse than the other samples. The phytin in the soy milk and isolate 1, although still present, reacted with the protein to a lesser extent, causing less lowering of solubility. The additional treatment shown in Figure 1 of isolates 2 and 3 with HCl or oxalate further denatured the protein (causing phytin to bind protein more extensively) caused more insolubility at the lower pH range than the less rigorous treatment used on the soy milk and isolate 1.

TABLE 3

THE NITROGEN DISPERSIBILITY VALUES OF AQUEOUS DISPERSIONS OF ISOLATED PROTEIN AND SOY MILK SAMPLES
(Percentage of Total Protein)

Product pH	Freeze-dried Soy Milk	Pro Fam 90 H/S	Soy Isolate 1	Soy Isolate 2	Soy Isolate 3
1.5	85.6	41.8	83.1	82.7	84.3
	85.1	41.3	83.2	85.1	81.9
3.0	82.2	40.1	82.9	81.5	86.4
	82.9	38.8	82.2	81.2	86.6
7.0	84.7	41.9	80.7	49.5	38.7
	84.7	42.4	80.9	51.4	39.8
9.5	86.2	78.2	84.4	83.4	81.0
	86.8	77.0	84.0	83.7	90.9

Freeze-drying the samples improved the dispersibility of all the laboratory produced samples since it is less harsh than spray drying. The problem with dispersibility was especially evident at pH 7.0, 3.0, and 1.5. At neutral and lower pH, the commercial isolate shown in Table 3 maintained a constant level of dispersible protein over this range. This can be attributed to the fact that the commercial isolate was a spray-dried product which had phytin bound irreversibly to the protein, giving the isolate a low dispersibility value.

The data in Table 3 show that the processing procedure that produced the isolated protein with the highest level of dispersibility over the spectrum of pH values was procedure 1. This advantage in suspendibility is very important, especially at pH 7.0 because this neutral point is where most isolates are dried and packaged for use.

Emulsion Capacity

Table 4 contains data on the emulsion capacities of the various isolated protein samples as well as liquid and freeze-dried soy milk. From the values in Table 4 and from the results obtained after rank analysis of the data was made, it can be concluded that isolate 1 is the superior emulsifier. Isolate 1 surpassed the samples in emulsifying ability by as much as twice. Fresh soy milk has a favorable emulsion capacity. This can be attributed to the fact that although the fresh soy milk has a lower

amount of protein present, all of the protein was in solution or dispersion. Additionally, lecithin is present in soy milk as well as saccharides and carbohydrates which could have a favorable effect on emulsion capacity.

TABLE 4

THE EMULSION CAPACITIES OF ISOLATED SOY PROTEIN AND SOY MILK SAMPLES

Product	Fresh Soy Milk		Freeze-dried Soy Milk		Pro Fam 90 H/S	
Trial	1	2	1	2	1	2
	(mls)		(mls)		(mls)	
Observations						
1	91.4	91.7	89.3	88.4	56.6	55.3
2	91.5	91.0	90.1	88.5	56.5	55.9
3	91.3	91.7	87.5	91.5	57.0	57.3
4	90.9	90.9	87.0	89.5	57.0	57.7
5	90.5	90.8	86.0	94.0	55.5	60.1
Rank Sum	22		28		52	

Product	Isolate 1		Isolate 2		Isolate 3	
Trial	1	2	1	2	1	2
	(mls)		(mls)		(mls)	
Observations						
1	123.8	133.1	61.5	65.0	54.7	56.2
2	128.5	132.2	60.8	64.1	56.4	55.8
3	132.0	134.1	58.5	60.9	56.7	56.9
4	129.1	136.3	61.5	66.5	56.5	58.3
5	124.5	135.3	65.9	61.0	56.1	56.9
Rank Sum	10		41		57	

Freeze-dried soy milk had a better emulsion capacity than isolates 2 and 3, but poorer than fresh soy milk and isolate 1. This can be attributed to the fact that it lost some of its protein solubility during freeze-drying, yet because of no additional treatment, the emulsion capacity was still higher than isolates 2 and 3. Lecithin and saccharides which favorably affect the emulsion capacity were still present because the soy milk underwent no additional treatment.

Isolates 2 and 3 had very poor emulsion capacities. This corresponds to the nitrogen dispersibility values discussed earlier. These values indicated that the lower the dispersible nitrogen, the lower the emulsifying ability of the protein. The Pro Fam 90 H/S showed a higher emulsion capacity than isolate 3, which was the reverse of the soluble nitrogen data. This can be explained in that the commercial isolate was a more finely divided powder than the other samples. The small solids aided in emulsification. Therefore, the commercial isolate, although more denatured and less dispersible, had a slightly higher emulsion capacity.

Electrophoresis

Figure 2 contains the electrophoretic data for the various samples used in this study. Although the electrophoretic patterns of each of the samples were expected to

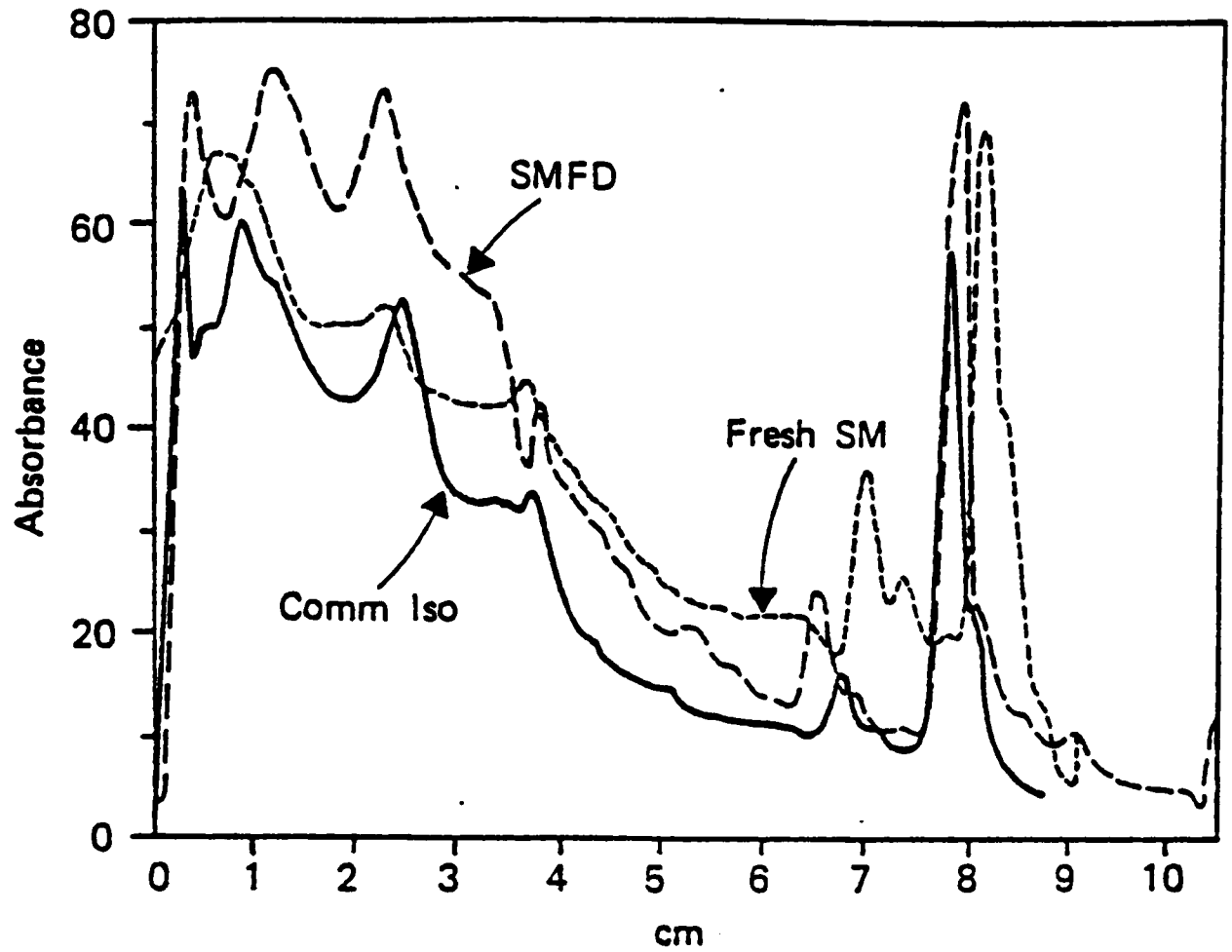


Figure 2a--SMFD, Soy Milk Freeze-Dried; Comm Iso, Commercial Isolate; Fresh SM, Fresh Soy Milk.

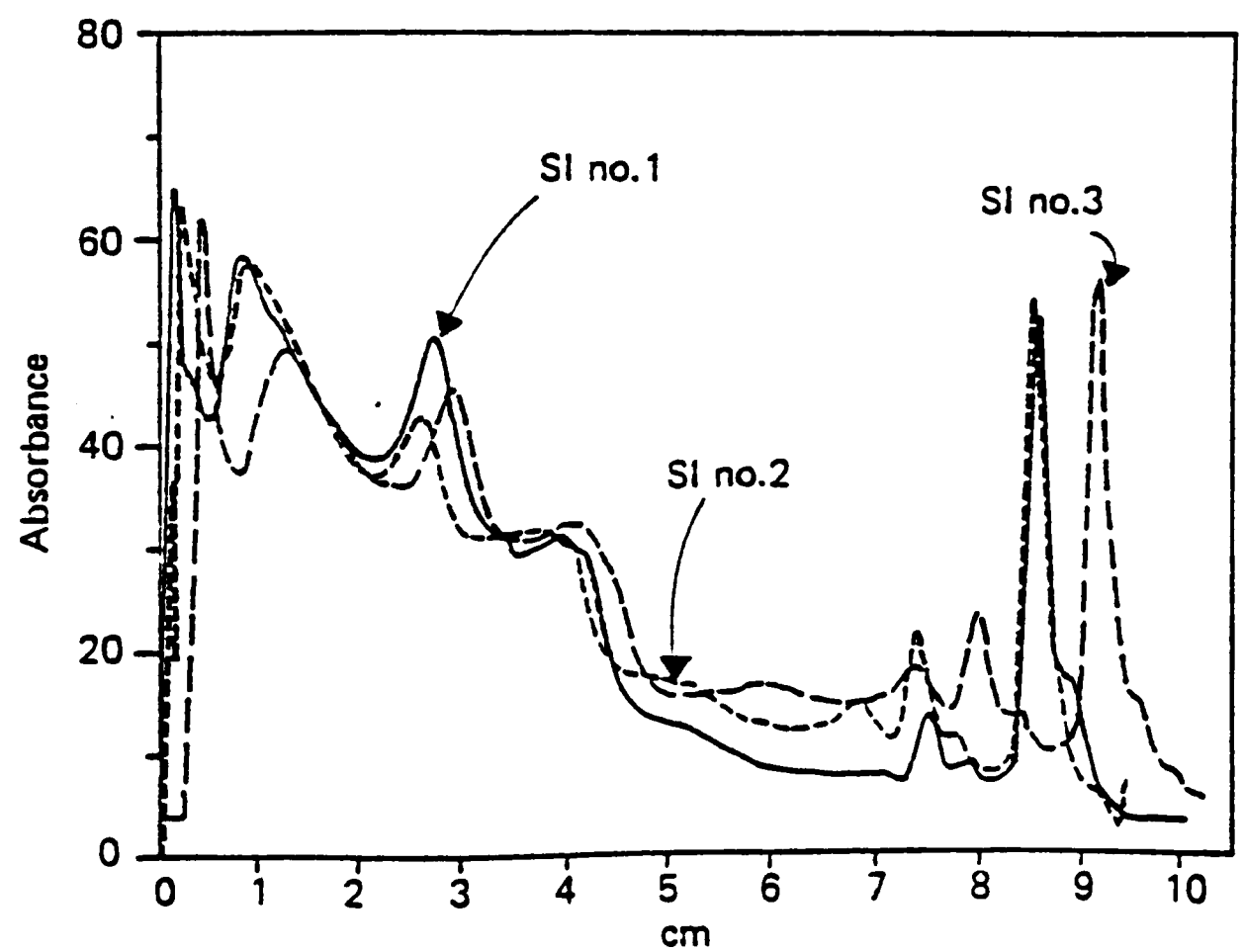


Figure 2b--Protein Absorbance Scans of Polyacrylamide Gel Electrophoresis Gels Obtained From Isolated Protein and Soy Milk Samples. SI, Soy Isolate.

contain proteins with different migration lengths, thus giving an indication of the ways in which the samples differ in composition, the patterns were quite different. The major difference in the bands is the intensity of the band. The majority of the protein is found in three peaks within the 0-4 cm range and another band in the 8-10 cm range. The 6-8 cm range contains very little protein components other than three small peaks. These graphs indicate that most of the protein components are highly insoluble, tending to migrate poorly through the gel, forming the three large bands in the 0-4 cm range. The rest of the protein is broken down into protein fragments and polypeptides which migrated further through the gel to give rise to the other peaks. From this data, it can be assumed that protein composition between all the samples was similar and thus does not provide information on the effect of different protein types on the dispersibility of soy protein. More electrophoretic study is needed to determine the nature of the proteins in the various samples and their effects on solubility.

CHAPTER V

SUMMARY

Current commercial soy protein concentrates and isolates exhibit limited water solubility and dispersibility, due in part, to the insoluble complexes formed between soy protein and phytic acid. By adjusting the pH of an alkaline suspension of defatted soy flakes to 3.0 rather than pH 4.5 or adjusting the pH of the suspension to 3.0 in the presence of calcium chloride, it has been assumed that an isolate of high yield and high dispersibility can be obtained. This study prepared pH 3.0, pH 3.0 treated with calcium chloride, and pH 4.5 isolates on a pilot plant scale and the differences in yield, nitrogen dispersibility, emulsion capacity, and electrophoretic patterns were studied.

It was found that pH 3.0 and pH 3.0 treated with calcium chloride isolates yield less than half the protein of the pH 4.5 isolate at pH 7.0. This was attributed to the phytate-bound protein being lost in the additional processing steps involved in producing the pH 3.0 and pH 3.0 treated with calcium chloride isolates. The pH 3.0 treated with calcium chloride isolate's yield was only

one-third as much as the pH 4.5 isolate. This lower yield was attributed to the more extensive treatment the isolate underwent.

The data associated with the nitrogen dispersibility test showed that the additional processing used on the pH 3.0 and pH 3.0 treated with calcium chloride isolate affected the dispersibility of the nitrogen adversely, rather than enhancing dispersibility. The pH 4.5 isolate showed high nitrogen dispersibility especially at pH 7.0 and illustrated that the additional treatment of the pH 3.0 and pH 3.0 treated with calcium chloride isolate affected dispersible nitrogen adversely by causing additional insolubility.

Emulsion capacity data for the pH 3.0, pH 3.0 treated with calcium chloride, and pH 4.5 isolates showed the pH 4.5 isolate to be the superior emulsifier. The lower protein solubility of the pH 3.0 and pH 3.0 treated with calcium chloride caused their poor emulsifying ability.

Polacrylamide gel electrophoresis data showed that the pH 3.0, pH 3.0 treated with calcium chloride, and the pH 4.5 isolates have similar protein composition and thus did not provide information on the effects of different proteins on dispersibility of soy protein isolates.

Further research could be directed toward a more detailed and intense electrophoretic study to determine quantitatively the types and amounts of each protein present in each isolate.

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APPENDIX A

PROCEDURE FOR MICRO-KJELDAHL DETERMINATION OF NITROGEN

This procedure is a verbatum transcript of AOAC (1975) method 47.021-47.023. Any deviation from the official procedure is noted.

47.021 Reagents

(a) Sulfuric acid.--Sp gr 1.84, N-free.

(b) Mercuric oxide.*--N-free.

(c) Potassium sulfate.*--N-free.

(d) Sodium hydroxide-sodium thiosulfate soln.**--Dissolve 60 g NaOH and 5 g $Na_2S_2O_3$ in H_2O and dil. to 100 ml or add 25 ml 25% $Na_2S_2O_3.5H_2O$ to 100 ml 50% NaOH soln.

(e) Boric acid soln.--Satd soln.

(f) Indicator soln.--(1) Methyl red-methylene blue.--Mix 2 parts 0.2% alc. Me red soln with 1 part 0.2% alc. methylene blue soln; or (2) Methyl red-bromo-cresol green soln.--Mix 1 part 0.2% alc. Me red soln with 5 parts 0.2% alc. bromocresol green soln.

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*Catalyst consisted of 3.2 g $CuSO_4$ and 96.8 g anhydrous sodium sulfate.

**60% sodium hydroxide was used instead.

(g) Hydrochloric acid.--0.02N. Prep. as in 50.011 and standardize as in 50.015 or 50.017.

47.022 Apparatus (9)

(a) Digestion rack.--With either gas or elec. heaters which will supply enough heat to 30 ml flask to cause 15 ml H_2O at 25° to come to rolling boil in $\geq$2 but <3 min.

(b) Distillation apparatus.--One-piece or Parnas-Wagner distn app. recommended by Committee on Microchemical Apparatus, ACS (9).

(c) Digestion flasks.--Use 30 ml regular Kjeldahl or Solty-type flasks (9). For small samples, 10 ml Kjeldahl flasks may be used.

47.023 Determination

Weigh sample requiring 3.10 ml 0.01 or 0.02N HCl and transfer to 30 ml digestion flask. If sample wt is <10 mg. use microchem. balance (max wt 100 mg dry org. matter). Use charging tube for dry solids, porcelain boat for sticky solids or nonvolatile liqs, and capillary or capsul for volatile liqs. Add 1.9±0.1 g K_2SO_4, 40±10 mg HgO, and 2.0±0.1 ml H_2SO_4. If sample wt is >15 mg, add addnl 0.1 ml H_2SO_4 for each 10 mg dry org. matter >15 mg. Make certain that acid has sp gr $\geq$1.84 if sample contains nitriles. (10 ml flasks and 1/2 quantities of reagents may be used for samples <7 mg.) Add boiling chips which pass No. 10 sieve. If boiling time for digestion rack

heaters is 2-2.5 min, digest 1 hr after all H_2O is distilled and acid comes to true boil; if boiling time is 2.5-3 min, digest 1.5 hr. (Digest 0.5 hr if sample is known to contain no refractory ring N.)

Cool, add min. vol. of H_2O to dissolve solids, cool, and place thin film of Vaseline of rim of flask. Transfer digest and boiling chips to distn app. and rinse flask 5 or 6 times with 1-2 ml portions H_2O. Place 125 ml Phillips beaker or erlenmeyer contg 5 ml satd H_3BO_3 soln and 2-4 drops indicator under condenser with tip extending below surface of soln. Add 8-10 ml NaOH-$Na_2S_2O_3$ soln to still, collect ca 15 ml distillate, and dil. to ca 50 ml. (Use 2.5 ml H_3BO_3 and 1-2 drops indicator, and dil. to ca 25 ml if 0.01N HCL is to be used.) Titr. to gray end point or first appearance of violet. Make blank detn and calc.

%N = [(ml HCL - ml blank) x normality x 14.007 x 100]/mg sample.

APPENDIX B

PROCEDURE FOR POLYACRYLAMIDE GEL ELECTROPHORESIS (PAGE) TECHNIQUES USED TO SEPARATE PROTEINS

The procedure for preparation of the PAGE gel is as follows:

Stock solutions--These solutions were prepared using distilled water and stored in brown glass bottles at refrigerator temperatures.

TABLE 5

STOCK SOLUTIONS

(A)	(B)	(C)
1N HCl 24 ml	1N HCl 48 ml	acrylamide 28.0 g
THAM 18.1 g	THAM 5.98 g	BIS .735 g
Temed 0.12 ml	Temed 0.46 ml	dilute to 100 ml
dilute to 100 ml (pH 8.8-9.0)	dilute to 100 ml (pH 6.6-6.8)	

(D)	(E)	(F)
acrylamide 20.0 g	Riboflavin 4 mg	Sucrose 40 g
BIS 5.0 g	dilute to 100 ml	dilute to 100 ml
dilute to 100 ml		

(G)
Ammonium persulfate 0.14 g
dilute to 100 ml

(H)	(I)	(J)
Buffer 10x(dilute 100 ml to 1 liter)	Gel Sample Stain 0.25 g Comassie Blue	Tracking Dye .005% 0.005 g Bromphenol Blue
THAM 3.0 g	45.5 ml CH_3OH	dilute to 100 ml
glycine 14.4 g	45.5 ml H_2O	
dilute to 1 liter (pH 9.2)	9.2 ml glacial acetic acid	

TABLE 6

PREPARATION OF GELS

Separating gel	Stacking and Sample gel
1 volume A	1 volume B
1 volume C	1 volume D
(pH 8.8-9.0)	1 volume E
Add 2 volume of G to gel	4 volume F
	1 volume distilled water (pH 6.6-6.8)
	To gel expose to flourescent light

Procedure

Glass gel tubes 12.5 cm long were washed with soap and water. The tubes were then submerged in Photo-Flo 200 solution (Eastman Kodak Co., Rochester, N.Y.) for approximately five minutes to insure easy removal of the gel. The tubes were allowed to dry well. Over one end of each gel tube a small piece of parafilm was stretched, over the parafilm a rubber serum cap was placed. The tubes were placed in a plastic polymerization rack. The separation gel was mixed according to Table 6. 1.7-2.0 ml of the gel was introduced slowly into each tube. 0.50 ml of distilled water was layered on top of the separation gel to insure a flat surface on the separation gel. The gels were allowed to solidify for approximately 20 minutes. The water layer of each tube was removed by tapping the tube on an absorbant tissue. The stacking and sample gel solution was prepared as in Table 6. Stacking gel solution (0.3 ml) was introduced to each tube. 50 ml of distilled water was layered

onto the top of the stacking gel. The gels were then exposed to a strong fluorescent light for 20 minutes to polymerize the stacking gel. The water layer was removed. The protein samples containing approximately 15 mg/ml were introduced into each tube. 0.3 ml of sample gel was introduced into each tube and mixed evenly with the sample. 50 ml of water was layered on the sample gel. Fluorescent light was used to polymerize the stacking gel (20 minute exposure). The gel tubes were placed into the electrophoretic apparatus.

Approximately 700 ml of solution H (pH 9.2) was introduced into the lower buffer reservoir of the electrophoretic apparatus. Each gel tube was filled with buffer solution to exclude air bubbles. The apparatus was lowered into the lower electrophoretic reservoir. Buffer solution H was layered into the top reservoir of the apparatus until all gel tubes were covered. 2.0 ml of Bromphenol Blue tracking dye was added to the upper reservoir. The top was secured to the upper reservoir and the two electrodes were attached to the power supply. The gel tubes were electrophoresed for approximately 3-1/2 hours, at which time the tracking dye was a few millimeters from the bottom of each tube.

The gels were removed from the apparatus. The gels were removed from the tubes by rimming the gels using

a needle, syringe, and a 50% glycerine solution. The proteins were fixed and stained in the gels by soaking each gel in the comassie blue stain for four hours. The gels were removed and destained for approximately three days in a Bio Rad Laboratories Model No. 172A Gel Electrophoresis Diffusion Destainer (Richmond, California) which contained 7% acetic acid.

The gel was removed from the destainer and stored in 7% acetic acid.