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dissertation entitled

Production and Functional Properties of High Protein Food Ingredients From Phaseolus Vulgaris

presented by

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has been accepted towards fulfillment of the requirements for

Ph.D. degree in Food Science

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Date December 20, 1988

PRODUCTION AND FUNCTIONAL PROPERTIES OF HIGH-PROTEIN FOOD INGREDIENTS FROM *Phaseolus vulgaris*

By

Andrew Lloyd Kohnhorst

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Food Science and Human Nutrition

1988

ABSTRACT

PRODUCTION AND FUNCTIONAL PROPERTIES OF HIGH-PROTEIN FOOD INGREDIENTS FROM PHASEOLUS VULGARIS

By

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Dry beans (Phaseolus vulgaris) are a good source of protein. However, per capita consumption has declined in the U.S. over the past several decades. The production of high protein materials which could be used as ingredients in fabricated foods would help increase consumption of the dry beans. This investigation was designed to determine the suitability of high protein materials from dry beans as food ingredients.

A lab scale air classification process for producing high protein flour from navy and kidney beans was developed. The protein content of the navy flour was 47% while that of the kidney flour was 50%.

Several methods were evaluated for production of concentrates from dry beans. Results showed the highest yield and protein content was achieved by an isoelectric precipitation method which yielded 83% protein.

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Functional properties of high protein flours and isoelectric precipitates from navy and kidney beans were compared with similar commercial soy products. Processing had no effect on the digestibility or methionine content of the high protein bean flours and precipitates. The high protein flours emulsified more oil and formed stronger gels than a similar soy flour and all bean products formed higher volume foams than soy products. Results indicated the functional properties of the high protein bean materials was similar to the soy.

The dynamic rheological properties of a navy protein concentrate (NPC) at 0.0-0.6M NaCl and pH 4.5-8.0 were determined during heating from 50°-94°C using a Fluid Spectrometer. The concentrate coagulated when no NaCl was added and at pH 4.5 but the protein structure was stabilized against denaturation at pH 8.0 and 0.6M NaCl. These results were similar to changes in 8-anilino-1-naphthalene-sulfonic acid (ANS) hydrophobicity and insoluble protein content during heating of NPC from 30-94°C.

ACKNOWLEDGMENTS

The author would like to thank Dr. M.A. Uebersax for serving as the Academic Advisor. He would also like to thank Drs. M.R. Bennink, G.L. Hosfield, P. Markakis, D.M. Smith, M.A. Uebersax, and M.E. Zabik for serving on the PhD Guidance Committee.

Thanks is owed to Dr. Bennink for his many hours of guidance in various aspects of the research program.

The author would also like to express a deep debt of gratitude to Dr. D.M. Smith for serving as the Thesis Advisor and for all of her help, guidance, and encouragement in both the final research study and in the final stages of this research program. The achievements of the this research program are very much the result of her patient guidance through the sometimes bizarre world of research.

Thanks are also due to the people who so warmly welcomed and accepted another "groupie" in their lab: Valente Alvarez, Bryan Bueschel, Tom Herald, and Rod Nuckles. Special thanks also go to Tom Herald for spending long hours helping to collect data from the Fluid Spectrometer. Thanks also go to Samir, Marce, Janice, Rosemary, Jaffer, Charlie, Zhang-hao, Nicki, Jo-An, S.-Y. Liao, Nirmal, and Mitch. Special thanks go to Tu for waiting and for having the patience to put up with me.

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I. INTRODUCTION

A. Introduction to Research

Dry beans (*Phaseolus vulgaris*) are important sources of protein in the Third World, especially in Central and South America. The beans are rich in lysine but are deficient in certain amino acids, most notably methionine (Carpenter, 1981). Another problem with beans is the presence of large quantities of anti-nutritional factors, such as lectins which limit the absorption of the proteins in the small intestine.

Michigan is one of the leading producers of kidney and navy beans in the United States. Over the past twenty five years, the per capita consumption of beans has declined considerably in the U.S. This has led to interest in new products which can overcome some of the anti-nutritional problems associated with beans and also provide new opportunities for development of new bean ingredients for fabricated foods (Aguilera et al., 1981). Two potential ingredients from dry beans are protein concentrates and isolates. These could provide for both the nutritional improvement of foods and also help impart desired functional properties in various food systems in the same ways that soy proteins have been used.

The Food and Nutrition Service of the USDA defines a vegetable protein concentrate as one which is not less than 65% protein (N x 6.25) but less than 90% protein. An

isolate is one which contains not less than 90% protein. These definitions have been used throughout this project.

Soy proteins have been used in a variety of food applications ranging from meat protein replacements to foods in which soy is the major ingredient, e.g. tofu and tempeh (Kinsella, 1979; Pearson, 1983). Soy protein has found wide-spread use in this country because soy beans are grown extensively in the United States and because the protein is relatively inexpensive when compared to other protein sources. These factors have led to large scale production of soy protein concentrates and isolates and their use in food systems in the U.S.

B. Note About Organization of Dissertation

The research in this dissertation has been divided into three studies with several common sections of text arranged around them. The first section is a common review of the literature for the entire dissertation. This is followed by a section which describes the rationale for the entire study along with the objectives for each study. The next section is a detailed Materials and Methods section in which every method used in the entire dissertation is described in detail along with the study in which it was used.

The next three chapters consist of the three studies which comprise the dissertation research. The first is

concerned with the evaluation of methods for production of high protein flours and concentrates from dry beans, the second with the determination of the functional properties and the effects of processing on high protein flours and isoelectric concentrates of dry beans, and finally the third is about the effect of pH and NaCl concentration on progel formation as determined by dynamic rheological properties of a navy protein concentrate. It should be noted that each of these studies is organized using the format of the Journal of Food Science: it contains a short introduction and literature review, an abbreviated materials and methods section (which repeats to a certain extent the more detailed methods of the independent Materials and Methods section), and finally a results and discussion section.

The final major section of text in the dissertation is a common conclusions section in which the findings from each study are summarized. This section will enable the reader to find the conclusions from each study much faster than would be possible without it. Finally, there is a common reference section for the entire dissertation followed by several appendices.

II. REVIEW OF THE LITERATURE

A. Characteristics of Dry Bean Proteins

Common beans contain 20-30% protein on a dry weight basis of which 55-80% are globulins and 10-20% are albumins (Chang and Satterlee, 1982). The amount of protein in a bean varies among varieties. For example, Pusztai et al. (1979) investigated the protein content of ten varieties of kidney bean and discovered that the protein content ranged from 23% for Prelude to 31% for the Cornell variety. The amount of globulins present (as a per cent of total protein) varied from 62% for 251, Cornell, 222, and 240 down to 55% for Prelude and 196.

Deshpande and Nielsen (1987) investigated the nitrogenous constituents of four legume species and their varieties. The total nitrogen of whole seeds ranged from 3.2-4.2% of which 8.3-14.5% was non-protein nitrogen. Water and salt soluble proteins were 72-94% of the total seed protein with mean protein contents of 67 and 87% and carbohydrate contents of 17-30% and 4-14% respectively. The sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) patterns for *Phaseolus* proteins were more complex than those of other legume species.

Koehler et al. (1987) investigated the protein content and protein digestibility of thirty-six varieties of eight types of dry beans for protein content and protein quality as determined by *Tetrahymena pyriformis* on

the raw bean flour. The amount of protein ranged from 17.5% for the Pinto variety NW-590 to 28.7% for the red kidney variety Royal Red. Except for kidney beans, there was little varietal difference in protein content. Pinto beans had the highest protein quality with six varieties having values of 90 or more and the rest having values of 80 or more. Red kidney beans had the lowest protein quality with a mean value of 59. Thus, there can be a great deal of variability between different varieties of the same type of bean.

The proteins in beans can be classified into two categories: metabolic and storage proteins. The storage proteins tend to be found in the globulin fractions while the metabolic (enzymatic or non-storage proteins) are primarily found in the albumin fraction (Deshpande and Nielsen, 1987). The storage proteins are more important in the study of protein functionality since they are a higher percentage of the protein in the seeds and "are responsible for many of the characteristics of the seed protein fraction" (Boulter, 1981). The storage (reserve) material serves to provide a source of nitrogen and carbon compounds for the seedling. The nitrogen is provided as protein while the carbon is in the form of oil and/or starch (as is the case with dry beans) (Derbyshire et al., 1976). Several recent studies have shown that the reserve proteins of dry beans are synthesized on the rough endoplasmic reticulum and later accumulated in the protein

bodies (Bollini and Chrispeels, 1979; Bollini et al., 1982). It can be arbitrarily assumed that extracted proteins which are more than 5% of the total protein in a seed are storage proteins (Derbyshire et al., 1976). Beachy (1982) extensively reviewed molecular studies which helped define the factors controlling the biosynthesis of seed protein in soybeans, peas, french beans, and several varieties of legumes while methods used for the isolation and characterization of legume storage proteins were extensively reviewed by Derbyshire et al.(1976).

The isolation and characterization of legume proteins in general and dry bean proteins in particular has been the subject of a great deal of research in recent years (Pusztai and Watt, 1970; McLeester et al. 1973; Ishino and Ortega, 1975; Barker et al. 1976; Derbyshire and Boulter, 1976; Hall et al., 1977; Ma and Bliss, 1978; Chang and Satterlee, 1981) and the work has been summarized in several very extensive reviews (Derbyshire et al., 1976; Chang and Satterlee, 1982; Sgarbieri and Whitaker, 1982; Sathe et al., 1984;).

The two major reserve proteins of dry beans are vicilin and phytohemagglutinin which account for 50% and 10% respectively of the total protein content of the cotyledon at maturity (Bollini and Chrispeels, 1978). Lesser amounts of another protein, legumin, (an 11S protein) have been reported in dry beans (Derbyshire et al. 1976). McLeester et al. (1973) extracted globulin

fractions from *Phaseolus vulgaris* and *Vica faba* and subjected them to SDS-gel electrophoresis. This resulted in differing peptide band patterns for these two plants and led the authors to question the usefulness of the terms "legumin" and "vicilin" in the naming of dry bean proteins. The vicilin fraction (which they called G2) appeared to be a non-homogeneous group of proteins.

Investigators at the University of Wisconsin developed the terms G-1 and G-2 to describe the major globulins of dry beans since the features of these proteins don't always correspond to the terms legumin and vicilin as defined by T.B. Osborne (Ma and Bliss, 1978). The G-1 fraction was identified by these investigators as corresponding to legumin (7S protein) while G-2 was identified as corresponding to vicilin (McLeester et al., 1973) although there has been some disagreement between researchers about the correct nomenclature (Bollini and Chrispeels, 1978). Bollini and Chrispeels suggested that the 7S protein (vicilin) was identical to the G-1 proteins while phytohemagglutinin is comparable to what the Wisconsin researchers identified as the G-2 proteins. Also, the term Phaseolin is now used as the trivial name for vicilin (the 7S protein) (Brown et al., 1981b).

Phaseolin (vicilin) is a 7S globulin which forms an 18S configuration at pH 4.5 and has been reported to have between three to five subunits ranging in size from 23,000 to 56,000 (Pusztai and Watt, 1970; Derbyshire et al.,

1976; Bollini and Chrispeels, 1978; Brown et al., 1981a). Dieckert and Dieckert (1985) reviewed the available literature for seed storage proteins in general and concluded that the 7S proteins appear "to be dimers or trimers of the fundamental subunits" and that disulfide bridging between the subunits generally does not occur. However, "the native monomers seem to form associating-dissociating systems depending on the milieu." Very little amino acid sequencing data has been published for these proteins so that it is very hard to determine inter-species homology. Derbyshire et al. (1976) summarized the forms of the 7S proteins from different legume sources for which the characteristics have been determined. Each of these proteins has been reported to have one or two characteristic subunits. However, according to these authors, the picture is still very confused and much work needs to be done to clarify the structure of these proteins. The 7S globulin in dry beans has been determined to be a glycoprotein which contains 4.5% neutral sugars and 1.1% hexosamine (Pusztai and Watt, 1970). Chang and Satterlee (1981) isolated and characterized the major protein of Great Northern Beans using classical isolation techniques. The major protein subunits were found in the globulin fraction and had molecular weights of 51 and 45 kd (kilodaltons) with a total molecular weight that was estimated to be 188 kd which would make it a 7S protein. This protein was found

to account for 37% of the protein present in the crude bean extract and 31% of the total seed protein. This globular protein was a glycoprotein which contained 6.5% sugar (as glucose) and about 50% α-helix. The protein was most stable at phs between 4.0 and 6.0 and had a compact structure that was resistant to proteolysis. Nesser et al. (1985) investigated the carbohydrate moieties of isolated glycoasparagines from glycoprotein II (this is probably phaseolin) of white kidney beans. They showed that the structures were similar to oligomannoside-type chains found in glycoproteins from various sources.

Phytohemagglutinin (PHA), the lectin of dry beans, has been described (Bollini and Chrispeels, 1978) as a 6.4S protein with two subunits of molecular weight 34 kd and 36 kd. Estimates of PHA's molecular weight range from a low of 115 kd to a high of 150 kd (Coffey, 1985). Coffey (1985) reviewed seven different studies on the amino acid composition of PHA; and concluded that PHA had a large amount of aspartic acid and serine but had practically no cysteine and methionine.

Felsted et al. (1981) investigated the subunit composition of the PHA of kidney bean. These studies revealed that there were five isomers of PHA present. These isomers, in turn, were composed of different combinations of an erythrocyte reactive subunit (E) and a lymphocyte reactive subunit (L):E_4, E_3L_1,E_2L_2,E_1L_3,L_4.

SDS-PAGE showed that E_4 and L_4 were single proteins with molecular weights of 31.7 and 29.9 kd, respectively.

Einhoff et al. (1985) have shown that Leguminosa lectins will bind both the storage proteins and the glycosidase enzymes and that both the lectins and the lectin-bound proteins are found in the protein bodies. They suggested that lectins act during maturation of the plant to contribute to an orderly arrangement of the storage proteins in the protein bodies.

Legumin (which has been referred to as the G-2 protein) is less well characterized than the other two, but appears to have a sedimentation coefficient of about 11S and has a molecular weight of 340 kd. It is a glycoprotein which has an isoelectric point of pH 4.7 (Derbyshire and Boulter, 1976).

The occurrence of anti-nutritional proteins (including PHA of dry beans) of both legumes and cereals, their physical and chemical properties, and their physiological significance in both plants and humans has been reviewed by Gatehouse (1984).

Bollini and Chrispeels (1978) confirmed that vicilin and PHA are reserve proteins for the seedling. Seedling growth over a period of 11 days was accompanied by a decrease in the amount of both proteins. The amount of the original polypeptide decreased while the proportion of smaller molecular weight polypeptides increased. Isolated protein bodies from the beans were found to contain the

two subunits of PHA, the three subunits of vicilin, and a major polypeptide with a molecular weight of 60 kd.

Dieckert and Dieckert (1985) used amino acid data from various sources to statistically compare the relatedness of pairs of proteins each from a different seed using the difference index (DI). The critical significance values for this index were developed by Cornish-Bowden (1980). A strong test indicates a "negligible danger of indicating relatedness incorrectly while a weak test indicates significance at the 10% confidence level. The critical values are dependent on the number of residues being compared.

Using the DI, Dieckert and Dieckert (1985) were able to show that generally all of the basic and acidic subunits of legumins from various sources pass at least a weak test for relatedness while often they can pass a strong test. They also compared amino acid composition data from convicilin of peas, α and α' subunits of β-conglycinins of soybeans and α-conarachin of peanuts with that from the legumins. All except α-conarachin and β-conglycinin pass a weak test for relatedness. However, data is lacking to test for relatedness of vicilin proteins in the range of 45-55 kd across species. Thus it is possible that the major reserve proteins of vegetable seeds have a common ancestry. Also, it is possible to predict structure of unknown seed proteins from proteins with similar physical characteristics in species where the

structure is already known. However, the results of McLeester et al., (1973) indicate distinct differences between varieties of beans.

Dieckert and Dieckert (1985) noted that most vicilin-type proteins are glycoproteins. They speculated that the presence of covalently-bonded oligosaccharides may not be a requirement for biochemical functionality. Rather, this structure may be a relic of an ancestral protein which did require a structure of this type.

Millerd (1975) has extensively reviewed the biochemistry of legume seed proteins, while Higgins (1984) reviewed the synthesis and regulation of the major proteins in seeds including legumes.

B. Production of Protein Concentrates and Isolates

Over the past twenty years, there has been a great deal of interest in the production of protein concentrates from dry beans and other varieties of legumes. This research was done to improve the nutritional composition of commonly consumed foods or to provide a functional ingredient for formulated foods.

1. Isoelectric Precipitation

One of the commonly used methods for producing concentrates has been solubilization of legume flour in an alkali solution (usually pH 7.0-10.0). The solution is

centrifuged and the proteins isoelectrically precipitated at pH 3.5-4.5. The protein is washed several times in distilled water and may be neutralized back to pH 7.0. The resulting solution is then freeze-dried.

Isoelectric precipitation is also a common method for the production of protein isolates from soybeans (Meyer, 1971; Kolar et al., 1985). Different methods for the production of protein isolates from soybeans have been reviewed by Kolar et al. (1985) while those used for the production of concentrates have been reviewed by Campbell et al. (1985).

Fan and Sosulski (1974) used an isoelectric precipitation method to produce isolates from nine different legume flours including dry beans. The authors produced isolates which ranged from around 92-93% protein for soybean, lupine, and fababean to 82-83% for pea bean, lentil, and chickpea. The proteins of the two beans varieties are very soluble at alkaline pHs and tend to become more soluble as the pH increases (Evans and Kerr, 1963; Hang, et al., 1970) Thus a pH of 8.0 will extract and solubilize the proteins. According to Fan and Sosulski (1974), a pH of 11.0 will solubilize more of the proteins remaining in the flour mixture. The two centrifugation steps remove most of the plant material which is insoluble at the high pHs. The solution is further purified by reducing the pH to 4.5 which is the point of minimum solubility for the proteins of the two

varieties of beans (Evans and Kerr, 1963; Hang, et al., 1970). The proteins are precipitated, separated from the soluble material by centrifugation, and finally freeze-dried.

Kon et al. (1974) produced legume powders from California small white beans and pinto beans. The beans were ground and added to 8 volumes of water which was acidified to produce a slurry of about pH 3.5. The solution was boiled during the addition of the bean flour to prevent clumping. After cooking for 15 min, the slurry was neutralized to pH 6-7 and dried on a drum drier. This powder was referred to as the "acidified" powder. A "regular" powder was also produced in which beans were soaked and cooked for 1 hr in 4 volumes of water, slurried, and then dried on a drum drier. The two powders had almost identical bulk densities after grinding. Kon et al. (1974) found that the viscosity of a slurry from the acidified powder was less than that for the regular powder. The viscosity of regular powder slurries decreased rapidly with dilution, but the acidified slurry retained its viscosity at lower concentrations. Nutritional qualities of both bean powders were similar to those of whole cooked beans as determined by weight gain, protein efficiency ratio (PER) values, and digestibilities. Both powders were free-flowing, non-hygroscopic, and convenient to use.

Alli and Baker (1980) isolated proteins from dry beans using a citric acid extraction at various phs and also using the alkali extraction method of Fan and Sosulski (1974). The citric acid tended to produce bipyramidal crystalline and spheroidal structures in the concentrate while the sodium hydroxide produced amorphous structures when examined under a light microscope. Alli and Baker (1981) examined the structures of proteins isolated by citric acid and sodium hydroxide under the Scanning Electron Microscope (SEM). Several types of particulate structures were present in the citric acid extracted proteins while the sodium hydroxide proteins had only a coral-like structure.

Musakhanian and Alli (1987) produced protein concentrates from white kidney, navy, and lima beans using the isoelectric precipitation method of Fan and Sosulski and also precipitated the proteins using citric acid at pH 5.5 and 3.5. They then determined the molecular weights of the protein fractions obtained from the extract, the precipitate, and the supernatent of the precipitate of both the citric and the alkali extraction. They found that both the alkali and the acid extracts had a fraction of 230 kd which was the major protein component of the precipitate. The lower molecular weight fractions tended to be found in the supernatent, although the precipitate of the navy bean contained an 18 kd fraction which was present only in trace amounts in the kidney precipitate.

There were no major differences between the composition of protein fractions which had different structures under the microscope.

Musakhanian and Alli (1987) found that the lima bean was different from the other beans. The acid extraction gave a precipitate which had a molecular weight of 700 kd while the alkali extracted proteins had a predominant molecular weight of 270 kd. Since the acid extracted proteins had a bipyramidal crystalline structure while alkali extracted proteins had an amorphous structure, it was suggested that there was a difference in composition between proteins with different structures. The authors concluded that the type of extracting agent used in the aqueous extracts from which the proteins precipitate does not appear to affect the composition of the precipitated proteins.

Coffman and Garcia (1977) produced a protein isolate from mung bean flour using a process which involved extraction with 0.001 N NaOH, precipitation at pH 4.5, neutralization of the precipitate and freeze-drying. The isolate demonstrated good nitrogen solubility, foamability, and gelation but only fair buffering capacity. The isolate amino acid composition was similar to mung bean flour except for cystine which was destroyed during isolate preparation.

Summer et al. (1979) used alkali extraction followed by isoelectric precipitation to produce isolates from

field peas. Generally, the isolate which was neutralized back to pH 7.0 had better functional properties than the non-neutralized isolate.

Vose (1980) used an aqueous extraction method followed by isoelectric precipitation to achieve isolates containing 91% protein from field peas and horsebeans. Isolates of 90% protein from field pea and 94% from horsebeans were obtained by ultrafiltration.

Hsu et al. (1982) studied the effect of germination on the physicochemical and bread-baking properties of yellow pea (*Pisum sativum*), lentils (*Lens culinaris*), and fababeans (*Vica faba*). These authors used an isoelectric precipitation method to prepare the protein isolates. Ten percent dispersions of legume flours were made in distilled water, adjusted to pH 8.5 with 1N NaOH, and mixed for 30 min at 40°C. The extract was centrifuged at 220 x g for 15 min at 10°C and the extraction procedure repeated to increase the yield. The extracts from both isolations were combined and the pH adjusted to 4.5 with 1N HCl to precipitate the proteins. The mixture was centrifuged at 1570 x g for 10 min and the precipitates dispersed in water, adjusted to pH 7.5 with 1N NaOH and dialyzed for 24 hr at 10°C. The dialyzed protein was lyophilized. SDS-PAGE revealed that the number of small subunits increased following the germination of pea and lentil but only slight changes were evident for the fababean. All of the proteins had a relatively broad

isoelectric pH range between 4.0 and 6.0. Germination had no effect on the isoelectric point.

The emulsification ability of the protein isolates prepared by Hsu et al. (1982) was low at pH 6.5, but improved at pH 7.5. This was attributed to increased protein solubility as the pH increased above the isoelectric range of the proteins. Emulsification capacity of all the isolates increased following germination with fababeans the greatest increase. Protein isolates from germinated legumes showed increased foam capacity and decreased foam stability compared to the ungerminated samples. When protein concentrates were prepared from ungerminated seeds, only the lentil concentrate formed a gel. However, germination caused the fababean to form a gel which had a 12-fold increase in viscosity over the gel formed by the isolate from ungerminated seeds while there was no effect on the concentrates made from the other two legumes.

Dench (1982) prepared protein isolates from winged bean (*Psophocarpus tetragonolobus* (L.) DC) flour and investigated their functional properties and potential for use in human or animal foods. The isolates were prepared by solubilizing winged bean flour in distilled water and adjusting the pH to either 8.0 or 10.0 followed by centrifugation. The protein was precipitated by adjusting the pH to 4.5 followed by neutralizing the precipitate at pH 7.0. The isolates showed high dispersibilities and

formed heat stable emulsions. However, they did not form gels except in the presence of $CaCl_2$ and formed unstable foams. The isolates had high fat absorption values which were due in large part to their low bulk densities.

Flink and Christiansen (1973) investigated the use of *Vica faba* (faba beans) for the production of a protein isolate. They found that the optimum conditions for the isolate production were: pH of 8-10, particle size which is less than or equal to 0.2 mm, and extraction time of 10 min and a solvent:bean ratio of 5:1. Only the solution pH and the bean particle size had a big influence on the amount of protein extracted. The parameters which gave optimum protein extraction in the lab also gave the same results when tested in a pilot plant.

King et al. (1985) prepared lupin protein isolates by alkaline extraction and precipitation at pH 5.1, 4.2, and 4.9. The functional properties of these isolates were investigated and compared with those of soy isolates. Lupin protein isolates had better solubility and emulsification capacity when compared to the soybean isolate. Swelling and gelation properties of the lupin isolate were inferior to the soy unless the isolates were heated to 100°C before drying. This heat treatment improved the functional properties of the lupin significantly. These results indicated that lupin isolates could be substituted for soy isolates which is of

particular interest to countries such as Chile where lupin is extensively cultivated and soybeans do not grow well.

Naczk et al. (1986) evaluated the functional properties and phytate content of pea protein concentrates produced by a multistep solubilization at pH 2.5-3.0 followed by centrifugation. The functional properties of the pea concentrates were then compared with those of gluten and soy proteins. The phytate content of both gluten and soy concentrates were higher than the pea concentrates. The phytates tended to be associated with the proteins. The pea proteins were more soluble in water than either the soy concentrate or the gluten. It also absorbed more water than the gluten but less than the soy. The emulsification properties of the pea proteins were comparable to soy.

Thompson (1977) used an isoelectric precipitation method to prepare protein isolates from mung beans (*Phaseolus aureus*). Mung bean flour was extracted at pH 9 and 25°C for 20 min using a 1:15 flour to solvent ratio. The solution was then centrifuged for 20 min at 1000 x g. The pH of the supernatent was adjusted to 4.0 with 1N HCl and the solution was re-centrifuged. The residue from the first centrifugation was re-extracted at pH 11.0 and the proteins precipitated at pH 4.0 as before. Both of the resulting precipitates were washed with water, neutralized with 1N NaOH, and then freeze-dried. The isolate from the first precipitation was designated MI-I while that from

the second was designated MI-II. MI-I had a broader solubility curve and slightly better amino acid profile than MI-II. MI-I contained 92% protein (on a moisture free basis) and a high lysine content, but was limiting in methionine and cystine. When added as a protein supplement to wheat flour in bread, it caused decreased dough and bread quality. Breads supplemented with MI-I at the 10% level had a PER of 73% and were judged to be acceptable. They also had a 41% higher protein content than the unsupplemented control bread. Thompson (1977) reported that the high nitrogen solubility of MI-I indicated it would have good functionality in a variety of food systems.

Molina et al. (1976) used a single step extraction at pH 9.0 or a two step extraction method at pH 6.8 followed by isoelectric precipitation to produce protein concentrates from black-eyed peas (*Vigna sinensis*). The protein extraction efficiencies were 87% for the one step and 86% for the two step method. Both of the protein concentrates had protein concentrations which fluctuated between 66 and 70%. The concentrates also had a higher methionine and cystine content and a higher PER value than the original pea meal. Results indicated that the concentrates were suitable for use in pasta, sausages, and tacos.

Murray et al. (1981) produced a protein isolate by a method which was designed to be simple and less harsh than

other available methods. A high-protein fababean flour (52.9% protein) was placed in an aqueous solution at a concentration of 15% (w/v). This solution was then extracted using 0.3M NaCl and centrifuged at 5000 x g to remove starch granules and other cellular debris to yield a high salt-protein extract (HSPE). The HSPE was diluted with tap water and immediately formed a "milky-white system". This system was allowed to remain stationary in the dilution vessel for about 30 min during which time the white mass settled to the bottom as a viscous mass. Examination of the white mass under the light microscope showed that it possessed many of the characteristics of micelles, therefore, the authors referred to the final product as the protein micellar mass (PMM). The PMM contained 95.6% protein (N x 5.85) and no fiber or lipid, and 2.8% ash; PAGE of the PMM revealed two protein fractions of molecular weight 340 kd and of 140 kd which probably corresponded to the vicilin and legumin. A high heat absorption value was obtained by Differential Scanning Calorimetry (DSC) for the PMM at pH 6.0 as compared to the values obtained for an isoelectric precipitate extracted at pH 11.2, indicating that most of the proteins in the PMM were in the native state. Murray et al. (1981) also found that the PMM would form an elastic fiber when injected through a small orifice into hot water. The PMM was also found to possess a binding strength similar to egg white. The authors concluded that

PMM functionality was very different from conventionally produced isolates and that it offered a processing advantage because of the simplicity of the process.

2. Salt Extraction

Satterlee et al. (1975) produced a flour from Great Northern Beans and then extracted the proteins overnight in 2% NaCl. This was followed by centrifugation at 9000 x g and dialysis of the supernatent for 48 hr. The precipitated proteins were then centrifuged at 9000 x g and the pH of the supernatent lowered to 3.5. The supernatent was considered to be the albumin fraction while the precipitate was the globulin fraction. These fractions were studied separately and were also recombined to form a bean protein concentrate.

Chang and Satterlee (1979) developed a process for producing a bean protein concentrate from a mixture of five varieties of cull beans. The concentrate was produced by extraction of bean flour with a 0.2% salt solution followed by precipitation at pH 4.0 at various temperatures. An optional step in this process involved heating the isoelectrically precipitated protein to 90°C which helped increase the yield. A PER study of the various concentrates indicated that the concentrate obtained by acid precipitation at room temperature had higher nutritional quality than the concentrate which was acid precipitated at 90°C.

Sathe and Salunkhe (1981b) fractionated bean flour using salt solubilization, dialysis, and finally freeze-drying. They were able to obtain a protein isolate, a protein concentrate, and the isolated albumins and globulins from the bean protein. The protein content of the concentrate was 85.44% while that of the isolate was 92.43%. They discovered that 0.5% Na_2CO_3, 5% K_2SO_4, 5% SDS, and 0.02N NaOH all solubilized 93.6g of Lowry protein per 100g of Kjeldahl protein. Sathe et al., (1984) believed this process could be modified to use ultrafiltration instead of dialysis.

SDS-PAGE of the bean flour, concentrate, and isolate produced by Sathe and Salunkhe (1981a) revealed the presence of 22, 14, and 11 subunits respectively. The same three products contained predominant subunits with molecular weights of 294 kd, 146 kd, and 135 kd, respectively. Isoelectric focusing indicated that the flour contained 15 subunits, the concentrate had 16 subunits, and the isolate had 11 subunits.

3. Ultrafiltration

Because of the severe processing conditions to which proteins are subjected during isoelectric precipitation, there has been a great deal of interest in preparing concentrates under milder conditions so as to improve their functionality. One method which has been used to prepare protein concentrates under less harsh conditions

is ultrafiltration. UF also improves the yield and also develops true protein isolates (i.e. those with protein contents greater than 90%) because of the retention of proteins which are ordinarily lost during isoelectric precipitation (Flink and Christiansen, 1973; Fan and Sosulski, 1974; Vose, 1980). Hensley and Lawhon (1979) and Lawson et al. (1981) have described the use of UF in the production of soy isolates while Olsen (1977) developed a continuous UF process for the production of fababean isolates. The process of protein concentration by the use of UF along with some of the process parameters has been reviewed by Lewis (1982).

Sathe and Salunkhe (1981b) used dialysis following extraction of dry bean proteins with 0.5% sodium carbonate to produce concentrates (85% protein) and isolates (92% protein) from Great Northern Bean Flour. Sathe et al. (1984) believed that this process could be modified to use ultrafiltration instead of dialysis.

Sathe et al. (1982) produced concentrates from winged bean using two successive long term (16 and 10 hr respectively) extractions at pH 10.0 followed by centrifugation. The resulting supernatents were dialyzed for 72 hrs against distilled water and freeze-dried. SDS-PAGE revealed 9 subunits in both the flour and the concentrate with molecular weight ranges of 27 - 380 kd for the flour and 14.2 - 143 kd for the concentrate. A higher amount of concentrate was required to induce

gelation than that reported for other legume concentrates but the winged bean concentrate had a relatively high emulsion capacity. Concentrates had lower foaming capacities than similar soy products. The concentrate had lower tannins and trypsin, chymotrypsin, and α-amylase inhibitory activities compared to bean flour.

Vose (1980) developed a process for the production of protein isolates from field peas and horsebeans using a hollow fiber UF system. The resulting isolates contained 90% protein (for field pea) and 94% protein (for horsebeans).

4. Air Classification

Another method for producing legume flours with high protein contents is air classification, in which whole legumes are milled into a flour and are then separated using an air classifier into a high starch fraction and a high protein fraction. Increased yields can be achieved with repeated milling and air classification (Vose et al., 1976; Tyler et al., 1981). However, the lipid content of the bean flour which is air classified must be low to prevent agglomeration of the flour and also the carbohydrates in the flour must be in the form of starch to achieve classification (Lillford, 1981). The process

of milling followed by air classification to obtain high protein flours has been reviewed by Sosulski (1983).

Cloutt et al. (1986) investigated the effect of starch granule size on the separation behavior of legume flours during air classification in an Alpine Mill using two varieties each of cowpea (*Vigna unguiculata*), fababean (*Vicia faba*) and pigeonpea (*Cajanus cajan*). Particle size was analyzed using a Coulter Counter and was determined as the cut point. The cut point was the point where the weight of particles entering the coarse fraction equaled the weight of particles which pass into the fines. Particles with weights below the cut point enter the coarse fraction while those above it enter the fine fraction. Cloutt et al. (1986) found that cut point dry weight yield and starch content of fines tended to increase as classifier speed decreased. The bean varieties which had the smallest starch granules tended to produce the fines with the highest starch contents. The authors concluded that there was considerable intervarietal variation in starch granule size distribution for some species and there would therefore be considerable differences in the characteristics of air classified fractions between different varieties.

Air classification has been used for the production of high protein flour from navy beans (Patel et al., 1980; Aguilera et al., 1981), California small white beans (Kon et al., 1977), and pea, northern beans, chickpeas, lima

beans, fababeans, field peas, mung beans, and lentils (Sosulski and Youngs, 1979).

Fleming et al. (1975) compared the gelation and thickening of flours, concentrates, and isolates from defatted sunflower, field pea, and fababean to those of soybeans. The isolates were produced by isoelectric precipitation and the concentrates were produced by air classification. The authors found that the fababean concentrate and isolate formed medium gels which were somewhat less firm than the soy gel, lacked elasticity but had very high viscosities. The field pea concentrate had similar gelling properties to fababean, but the isolate formed a soft gel with lower viscosity. They concluded that fababean and field pea concentrates and isolates may be useful in applications where commercially prepared soy protein with the ability to form gels is now used.

Tyler and Panchuk (1982) investigated the effect of seed moisture content on the air classification of field peas and fababeans. Reductions in seed moisture were accompanied by declines in the protein content of the flour and in the starch separation efficiency, while protein fraction yield and separation efficiency increased.

Sosulski and Sosulski (1988) pinmilled and air classified field peas (*Pisum sativum*) and small fababeans (*Vicia faba minor*) into both protein and starch fractions. Flours of the same legumes were also extracted using an

aqueous alkali procedure followed by isoelectric precipitation to form protein and starch fractions. The high protein fraction from the field peas had a protein content of 52.7% while that from the fababean contained 72.5% protein. The wet-extracted concentrates had protein contents of 87.7% for field pea and 94.1% for the fababean.

The air-classified protein fractions of Sosulski and Sosulski (1986) had Nitrogen Solubility Index (NSI) values which were about half that of whole legume flours. The wet-extracted concentrates were highly soluble under the conditions of the test (100% for fababean and 83.6% for field pea). The dry-processed protein fractions showed low water hydration and oil absorption values compared to the alkali-processed protein, but the two protein types had similar emulsification values for both of the legume varieties. All of the protein products formed strong gels when tested in the Visco-amylograph.

Sosulski and McCurdy (1987) investigated the functionality of flours, protein isolates, and protein fractions from field peas and fababeans. The protein fractions were prepared by air classification while the isolates were prepared by either alkali extraction followed by isoelectric precipitation for the field peas or acid extraction and isoelectric precipitation for the fababeans. The functional characteristics of these products were compared to similar soy products. All of

the proteins in flour and protein fractions were highly soluble at acid pH with a narrow range of insolubility at pH 4-5. Water holding and oil absorption increased as protein content increased for all products while oil emulsification properties were uniformly high among all products. The bean protein fractions showed excellent whippability and foam stability compared to the soy controls.

Sumner et al. (1979) examined the storage stability of pea flour and air-classified pea protein concentrate during a one year storage period at four moisture levels. The microbial counts generally decreased during the storage period while the NSI and water absorption properties were not adversely affected during storage. The authors concluded that the moisture content and storage temperature had the greatest effect on the storage stability of pea products.

Patel et al. (1980) fractionated navy bean flour in an air classifier and attained a yield of 34.7% for a protein concentrate containing 62% protein concentration. Aguilera et al. (1981) also milled and air classified navy bean flour into high starch and high protein fractions. The high protein fraction contained 43.1% protein. The high protein flours were found to function favorably in a wide variety of foods.

Kon et al. (1977) hammermilled and then air classified the flour from California small white beans and

obtained high protein flours containing 45% protein. Examination of the high protein flour under a light microscope indicated that most of the hull portion was concentrated in the coarse fraction and that there was very little starch in the protein fraction.

Sosulski and Youngs (1979) milled and air-classified proteins from eight different legumes and compared their functional properties with those of soybean and lupine flours. The pea, Great Northern Bean, chickpea, limabean flours and their protein concentrates gave higher values in the functional property tests while fababeans, field pea, mung bean, and lentil had higher protein fractionation in the air classification step than the other four legumes. These authors found that high values for oil absorption, emulsification, whippability, and foam stability were characteristic of the high protein fractions. The high protein flours from Great Northern Beans and pea beans had protein contents of 55 and 50% protein respectively, 3% and 4% lipid, and both types had 1.4% starch.

Tyler et al. (1981) used the procedure of Sosulski and Youngs (1979) to form high protein flours from several types of legumes, including Great Northern and navy beans. The percentage of the total flour protein recovered in the protein fraction was used as a measure of protein separation efficiency (PSE). Navy and Great Northern beans produced PSE values of 80% and 87%, respectively.

Sahasrabudhe et al. (1981) pin-milled navy bean cotyledons and air-classified the resulting flour in an Alpine microplex air-classifier. The protein rich fraction was 31-35% of the total by weight and contained 50.3-57.5% protein. Nitrogen solubility at pH 7.5 ranged from 81.1-83.0 depending upon the bean variety. The water hydration capacity values of the protein fractions did not vary significantly among varieties but were lower than other vegetable products. The amino acid composition was similar to that of whole beans. Lipids and oligosaccharides were concentrated in the protein-rich flour.

C. USDA Project on Bean Flour Production

A procedure for dry roasting of beans followed by milling and air classification to produce various flours from dry beans was developed by Aguilera et al. (1982a and 1982b). The flour production process involved three basic steps. First, dry beans were rapidly heated to 120°C in a solid-to-solid heat exchanger to inactivate anti-nutritional factors and stabilize flavor. The roasted beans were either pin-milled to produce whole flour or cracked by corrugated rollers to aid in the removal of hulls by air aspiration. The cracked cotyledons were pin-milled to a fine flour which was further air-classified into high protein and high starch flours. This procedure

was used to produce high protein flours from navy and pinto beans along with high starch and high fiber flours from the two bean varieties.

1. Characterization of the Flour Fractions

The characteristics of the Navy flour fractions produced by the method of Aguilera et al. (1982a and 1982b) were evaluated by Lee et al. (1983). The high protein fraction contained 7.0% moisture, 4.7% ash, 41.3% protein, 2.5% fat, and 4.5% dietary fiber. It was found that stachyose was the major oligosaccharide in the fractions followed by sucrose, glucose, and trace amounts of raffinose. The high-protein flour had the highest stachyose content.

The high protein flour from pinto beans produced by the methods of Aguilera et al. (1982a and b) contained 50.83% protein, twice the 23.67% protein found in the whole bean (Zabik et al., 1987). The NSI of the protein in the high protein flour was 61.96 as compared to that of 44.95% in the pinto cotyledon.

Tecklenburg et al. (1984) found that the phytic acid which occurs naturally in beans was concentrated in the protein fraction by the air classification procedure of Aguilera et al. (1982b). The authors also found a strong correlation between protein content and the amount of zinc, iron, potassium, and magnesium in the flours. The

correlations between the three suggested that the minerals were present as metallic phytates in the protein flour . Naczk et al., (1986) found that phytates were primarily associated with proteins in field peas. Adsorption of iron wasn't hindered by the presence of phytic acid when the high protein flour was fed to anemic rats (Zabik and Uebersax, 1986).

The effects of processing using the methods of Aguilera et al. (1982a and 1982b) for dry roasting, milling, and air classification on protein content, SDS-PAGE patterns, and *in-vitro* digestibility for both the navy and pinto high protein flours were evaluated (Zabik and Uebersax, 1986). Roasting decreased the protein content of selected fractions, however, the SDS patterns of the salt soluble proteins were similar in the raw and roasted flours. The protein digestibility of the starch and protein fractions for both types of beans were similar and analysis indicated the amino acid content of the different flour fractions was similar.

2. Storage stability

Uebersax et al. (1982) examined the storage stability of the whole navy bean flour and the various fractions produced by the methods of Aguilera et al. (1982a and 1982b). The equilibrium moisture content (EMC) of the whole flour decreased with increased roasting time and

temperature. EMC increased as the relative humidity of storage increased. Similar effects of roasting conditions on EMC were seen in the other fractions. In the high protein fraction, Uebersax et al. (1982) showed that the flours with the highest protein contents had the highest EMC, while the hull flour with lower protein levels had the lowest EMC at all humidity levels. In all flour fractions, the NSI and sugar content decreased with increasing relative humidity values. Generally, the NSI values for flours stored at 6 and 9% moisture were unchanged after 24 months at 20°C.

D. Functional Properties of Dry Bean Protein Products

Hermansson (1979a) defined functional properties as those "physico-chemical properties which give information on how a protein will behave in a food system." These functional properties reflect complex interactions between the composition, structure, conformation, physico-chemical properties of the proteins, other food components, and the nature of the environment in which these are associated or measured. Kinsella (1976) extensively reviewed the functional properties of proteins in foods and classified the functional properties of proteins which are important in food systems.

1. Theoretical Considerations of Protein Functionality

a.Gelation

Protein gels can be formed through the use of heat or divalent cations. A gel is an ordered state where intermolecular forces are balanced so that a regular network is formed. If the intermolecular forces are too few, water will not be entrapped between denatured protein molecules and a gel will not be formed. If there are too many inter-protein interactions or if the interactions occur at random, the protein will be coagulated (Hermansson, 1979b; Schmidt, 1980).

According to Hermansson (1986), there are two basic types of heat-induced protein gels: those formed by "random" aggregation and those gels formed by more orderly associations of protein molecules. A protein solution can form either type of gel depending upon the repulsive charges between protein molecules. The change from one type of structure to the other can take place within 0.1 pH unit (Hermansson, 1986). Randomly aggregated-type gels tend to be opaque while the gels which are formed in a more ordered state from small globular proteins can be completely transparent.

Aggregated gels with the finest structures are generally formed under conditions similar to those necessary to first form a gel (Hermansson, 1986). As conditions get farther away from this point, the gel

structure becomes coarser. To understand the structure and properties of these types of gels, it is important to understand those factors which promote or restrict aggregate growth, such as surface tension and net surface charge (Hermansson, 1986). Protein molecules will arrange themselves into linear strands to yield a true gel structure if there is enough of an energy barrier to prevent random aggregation. Dissociation, denaturation, and association of the protein molecules must be understood to elucidate gelation mechanisms (Hermansson, 1986).

The classic mechanism for the formation of a heat-induced gel is that first proposed by Ferry (1948). He suggested that gelation is a two-step process: first a denaturation step where the native protein is unfolded and, secondly, an association step where the long protein molecules interact at specific points to form a network which entraps large amounts of water. It is known that for gels to form, the second step of gelation must be slower relative to the first (Hermansson, 1979).More recently, Damodaran (1988) proposed a modification to this model based upon his studies of the soy 11S globulin during cooling. In this model both a reversible gel, a partially refolded progel state, and a coagulum formed by aggregation are all possible. If the extent of refolding in the protein can be controlled by pH or addition of various salts during cooling it is possible to improve the

gelation of globular proteins even at protein concentrations below the optimum for gelation. Chou and Morr (1979) have theorized that the transition from sol to gel and the ability of gels to hold water may represent the formation of polarized water multilayers by the disruption of hydrogen bonding between adjacent molecules by forces such as heat. This disruption allows water to interact with the now exposed amino and carboxyl groups. During cooling the amino and carboxyl groups on opposing protein molecules may form electrostatic interactions between themselves and also water which provides the structure necessary to immobilize the free water.

Divalent cations (usually calcium) can be involved in inducing gelation of protein solutions such as occurs in the manufacture of cheese or in the making of tofu. The mechanisms for gelation by divalent cations are less well defined (Schmidt, 1980) although there have been numerous theories and models proposed to describe the cheesemaking process.

Most determinations of gelation characteristics of proteins have involved heating the protein to a given temperature under specific conditions and then somehow measuring the "gel strength". With the development of dynamic testing equipment such as the Rheometrics Fluid Spectrometer, it has become possible to follow the course of gelation of a single sample over a range of temperatures. Food gels can be thought of as viscoelastic

materials. If the material was a perfectly elastic solid, it would respond immediately to stress and maintain its original deformation characteristics over an infinite amount of time (Mitchell, 1980). (An analogy for this would be the result of pinching a rubber ball between thumb and forefinger.) A liquid would spread out infinitely to accommodate the applied force and there would be no resistance from the material to the force (i.e. no response). A gel can be thought of as a condition which is intermediate between a liquid and a solid and requires a finite time to respond to the stress. Changes in gel structure occur as the length of time over which the force is applied increases (Oakenfull, 1987).

If the amount of spreading or deformation which the material undergoes can be controlled, then the response of the material to the applied stress necessary to maintain a constant amount of deformation (or strain) over time can be determined. For an elastic solid, the application of force over a relatively infinite amount of time will cause no permanent damage to the structure of the solid and there will be no change in the amount of stress needed (Mitchell, 1980). Viscoelastic materials will tend to require less force to maintain the amount of deformation of structure over time and will thus become more liquid in nature. Viscoelastic materials can also be divided into viscoelastic solids and viscoelastic liquids (Mitchell, 1980). A viscoelastic solid contains some permanent

crosslinks which remain firmly in place while in a viscoelastic liquid whole molecules move relative to each other (Mitchell, 1980). If stress is applied over time to a viscoelastic solid, the increase in deformation of the structure will eventually cease and will return to zero when the stress is removed. A viscoelastic liquid will display a permanent deformation after removal of the applied force (Mitchell, 1980).

Viscoelastic materials can be characterized by dynamic experiments in which a sinusoidally oscillating stress or strain is applied to the material. If a material is an ideal elastic solid, the stress will be in phase with the strain; for an ideal liquid, the stress will be 90° out of phase with the strain (Mitchell, 1980). A viscoelastic material will have a phase angle somewhere in between the two.

The terms G′ and G″ can be used to express the results of dynamic experiments. G′ is referred to as the storage modulus and is defined as:

$$G' = \frac{\text{stress component in phase with strain}}{\text{strain}}$$

This term defines the elastic properties of the material and indicates the solid characteristics of the material. G″ is referred to as the loss modulus and is defined as:

$$G'' = \frac{\text{stress component 90° out of phase with strain}}{\text{strain}}$$

This factor of measures the energy lost as frictional heat due to viscous flow within the sample (Beveridge et al., 1984)) and thus indicates sample viscosity.

Another factor which is often used to define the characteristics of the material being tested is G* or the complex modulus. This factor is defined as:

$$G^* = [(G'^2)+(G''^2)]^{1/2}$$

This factor is proportional to the total amount of force needed to deform the sample (Anonymous, 1986). This factor would be expected to increase as a sample gels during heating and becomes more like an elastic solid.

Another factor which can be calculated from G' and G" data is Tan δ or the "loss tangent". This factor compares the liquid/solid characteristics of a protein solution. In principle, as gel networks form, the sample will become more elastic in nature and G' values will rise while the Tan δ values fall (Beveridge et al., 1984).

b. Foaming

The ability of proteins to form stable foams is important in the food industry in diverse applications such as whipped toppings, candies, and meringues. The theoretical explanation of what happens during the lifetime of a foam has been described by several authors (Cumper, 1953; Kinsella, 1979; Cherry and McWatters, 1981; Aubert et al., 1986). The role of proteins in relatively

simple emulsion systems and foam systems has been extensively reviewed by Halling (1981).

Aqueous foams are typically 95% air and 5% aqueous solution. About 99% of the solution is water with the remaining 1% composed of surfactants such as proteins (Aubert et al., 1986). The first step in the formation of a foam is the denaturation of the protein at the air/water interface and the formation of a denatured protein monolayer. The rate at which a protein reaches an interface is related primarily to its diffusion coefficient (German et al., 1985). A surface composed mainly of water molecules is very rapidly covered by a thin monolayer of protein. Additional protein molecules are adsorbed only if they can do so without disrupting the integrity of the film monolayer. Disruption of the monolayer requires a protein which exhibits a tendency to bind to hydrophobic surfaces and is why hydrophobicity is highly correlated with foam forming ability (Townsend and Nakai, 1983). Gas is introduced into the monolayer and is entrapped by the proteins to form bubbles. Protein films on adjacent bubbles come in contact and trap liquid. When a protein is adsorbed at an air/water interface, the protein can minimize hydrophobic free energy by placing apolar side chains in the air or oil phase as an alternative to folding them into the interior of the molecule (Phillips, 1981). Initially in the development of a foam, the volume of liquid contained in the foam is

very high. This liquid layer between bubbles will readily drain away from the bubble structure, allowing protein groups to aggregate and weaken the surface film followed by the bursting of the bubble. If the viscosity of the solution is high enough, the liquid flow off the bubble structure will be retarded and the foam will be stable for a longer period of time. Also, the foam structure can be maintained if the protein denaturation and adsorption process and/or the bubble formation process is continued. Anything which serves to increase the viscosity of the protein solution or to denature the proteins in that solution will help to increase the stability of foams. Flexible proteins such as β-casein which can rapidly reduce the surface tension of the air/protein solution interface give good foamability, whereas a highly ordered globular molecule with a large number of highly structured regions, such as lysozyme or soy protein, is relatively difficult to surface-denature and gives poor foamability. German et al. (1985) found that the density of foams formed from both 11S, 7S, and a mixture of the two soy proteins was improved dramatically when intersubunit disulfides were cleaved with dithiothreitol. Likewise, Kim and Kinsella (1987) found that reducing intramolecular disulfide bonds with 5mM dithiothreitol enhanced the stability of foams formed by soybean glycinin (the 11S protein). The air cells in the foam formed by the more rigid protein were smaller than those in the foam formed

by the flexible protein, leading to differences in foam volume. Less flexible proteins form more stable foams probably because of the greater strength of gel-like adsorbed protein layers (Phillips, 1981).

c. Emulsification

Another important functional property of proteins is their ability to form stable emulsions. The formation and stabilization of emulsions is a complex subject and detailed descriptions of this process have been published elsewhere (Friberg, 1976; Becher, 1983)

An emulsion has been described as a system containing two immiscible liquid phases in which one phase is dispersed in the other as droplets (Nawar, 1985). The classic example of this is the oil and water system which is found in a salad dressing. In these systems, work must be applied to form small dispersed droplets and thus increase the interfacial area between the two liquids. Due to the large positive free energy at the interface of the two liquids, emulsions are thermodynamically unstable allowing coalescence or joining of fat globules and a reduction in interfacial area (Nawar, 1985).

In food systems, coalescence is usually prevented by the addition of emulsifiers. These are surface active agents that adsorb at the interface between the two phases of an emulsion and lower the interfacial tension,

preventing coalescence (Nawar, 1985). Proteins are surface active agents because they contain both hydrophilic and hydrophobic amino acid side chains and have been used extensively for emulsion stabilization.

Soy proteins have been extensively studied and used for their emulsion stabilizing ability in food systems, while other vegetable proteins have been studied to a lesser extent (McWatters and Cherry, 1981). Some of the foods in which soy has been used for emulsification include: sausages, soup, cakes, and bologna (Kinsella, 1979). Puski (1975) suggested that it is difficult to develop one model system to measure emulsification properties of vegetable proteins. He proposed that three model systems be developed to simulate three basic types of food emulsions: a comminuted meat sausage system, a low-fat milk system, and a high-fat mayonnaise system.

2. Functional Properties of High Protein Dry Bean Products

Sathe and Salunkhe (1981a) investigated some of the functional properties of a protein isolate, a concentrate, and the isolated albumins and globulins from Great Northern beans produced by salt extraction. These included: viscosity, gelation, emulsification ability, and foamability. The bean concentrate and isolate had comparable viscosities at corresponding concentrations, but were more viscous than the individual albumins or

globulins at the same concentration. This suggested that combining the albumins and globulins produced concentrates and isolates with different properties than the albumins and globulins had individually.

Sathe and Salunkhe (1981a) discovered that the lowest protein concentrations necessary to achieve gelation were: 10% for the flour, 8% for the concentrate, and 12% for the isolate. This suggested that gelation was not only a function of protein concentration but also the protein and nonprotein composition of the bean products.

The protein concentrates of Sathe and Salunkhe (1981a) were best at absorption of oil and water, while the flour was the worst. The isolate was very similar to the flour in both water and oil absorption capacity. The concentrate had the highest emulsion capacity and foam forming capacity among all three products and was better than egg albumen in foam forming ability. However, the stability of foams formed by the bean products was less than that of the egg. All of the samples showed increasing foamability as protein concentration increased to a maximum at 10% (w/v) solids.

Sathe and Salunkhe (1981c) investigated the functional properties of the bean products produced by salt solubilization, dialysis, and freeze-drying. The bean flour, concentrate, and isolate were found to have similar moisture sorption isotherms where maximum absorption occurred at 4°C and minimum absorption occurred

at 38°C. The equilibrium moisture content of the flour was higher than the other two bean products, possibly due to the presence of carbohydrates in the flour.

The protein products of Sathe and Salunkhe (1981c) had only modest buffer capacity at phs from 4.0 to 8.0. Modification of the proteins by succinylation or oxidation increased the oil absorption capacity of all three protein products and the water absorption capacity of the flour and isolate. However, modification of the concentrate decreased its water absorption. When the adhesiveness of the products was tested, that of the concentrate was found to be about half that of the flour while that of the isolate was approximately 1/8 that of the flour.

The amino acid composition and *in vitro* digestibility of bean flour and bean concentrate and isolate were evaluated (Sathe et al. 1981). The flour and the isolate had high amounts of acidic amino acids while the concentrate was high in hydrophobic amino acids. The sulphur amino acids and leucine were the first and second limiting amino acids respectively. All of the products had low *in vitro* digestibility but this was improved by heating with moist heat being more effective than dry heat.

The performance of the bean concentrate, isolate, and flour in cookies was evaluated (Sathe et al. 1981). It was noted that there was a decrease in cookie spread ratio as the protein content of the cookies increased.

Satterlee et al. (1975) determined both the emulsifying and whipping characteristics of the protein fractions and the concentrate produced by their salt extraction method. The emulsifying characteristics of the proteins were considered to be fair; the albumins had good emulsion capacity but poor stability, while the opposite was true for the globulins. The albumins had good foaming capacity and stability at a concentration of 5% and a pH of 4-5. The globulins weren't as good at foaming but did have slightly better foam stability than the albumins.

High protein navy and pinto flours were produced by pin-milling and air classification (Zabik and Uebersax, 1986). The amount of oil which could be emulsified by the flour decreased as the amount of flour increased (Zabik et al., 1987). This was attributed to the oil droplets becoming smaller as the amount of protein increased, resulting in a much larger surface area for protein emulsification.

The emulsification capacity of the navy bean protein flour was evaluated at various pH levels (Zabik and Uebersax, 1986). Results showed that emulsification capacity increased as pH increased from 3.2 to 8.0.

The pinto high protein flour produced stable high volume foams from pH 4-7 but the volume decreased significantly at pH 10 (Zabik et al., 1987). Foam viscosity increased with increasing pH, but increasing salt levels had no effect on foam volume or viscosity.

Foam drainage decreased significantly when the high protein flour was substituted for egg white. This was attributed, in part, to the water binding capacity of the starch in the flour.

3. Dynamic Testing of Rheological Properties

Very little work has been done to dynamically test the gelation characteristics of vegetable proteins. To date, most of the work has been conducted on egg or muscle proteins. As part of a larger study on the gelation of ovalbumin and soybean proteins, Van Kleef (1986) determined the gelation properties of a soy isolate and the 11S globulin using a Weissenberg Rheogoniometer as the materials were heated in solution from 50-98°C in water containing 25% isolate at pH 5.5. He noted that this solution is a thick paste at room temperature and has a G′ value. Upon heating, an increase in G′ was noted around 70°C followed by a second gelation step at about 90°C. Separate investigations indicated that the first increase was due to gelation of the 7S protein while the second was due to gelation of the 11S protein. The 11S globulin at pH 6.4 and room temperature had a measurable G′. There was no reason given for the difference in phs between the two samples. The G′ of the 11S protein decreased upon heating until about 60°C when a sharp increase occurred. There

was also a sharp increase in G′ as both the isolate and the 11S proteins were cooled.

Van Kleef (1986) also measured the effect of pH and ionic strength on G′ at room temperature for the soy isolate and the 11S protein. The soy isolate without salt displayed two G′ maxima at pH 4 and 6 and only one G′ maximum at pH 4.5 for an isolate solution containing 3% NaCl. The 11S globulin without salt displayed two maxima at pH 4.6 and 6.4 and one maximum around 4.5 for the solution containing 3% NaCl.

Beveridge et al. (1984) used a Weissenberg Rheogoniometer to follow the gelation of whey, soybean, and egg albumen solutions over time at one temperature. The soybean material was Promine D, a commercial soy protein concentrate, which was made up at various solids contents at the natural pH of the material (pH 6.8). The authors also performed SEM on pieces of protein gel. The G′ of the soy concentrate solution increased over time as both the heating temperature and solids content increased. Data indicated that G′ at 75, 80, and 85°C increased dramatically for the first 5 min and then tended to plateau or even tail off. Much the same trend was seen as the solids content of the mixture increased from 10.3 to 13.8%, although the G′ curves tended to be much smoother as the solids content increased. SEM micrographs of the soy gels showed them to consist of many large particles adhering together and apparently embedded in a

background matrix. Higher magnification revealed broken and collapsed proteinate spheres cemented together by a limited amount of gel matrix which was presumably formed by the heat induced gelation of the soluble proteins.

Kamata et al. (1988) used a Rheoner Creep Meter to analyze the creep behavior of soy glycinin under compression at different temperatures. From this, they were able to estimate the forces contributing to the structure of the gels. The curves could be described by a five component model which included an elastic, viscous, plastic, and two viscoelastic components. The elastic and the plastic components were found to contribute the most to the deformation behavior of the gels. Kamata et al. (1988) showed that the plastic component was the most important at temperatures from 10-50°C while the elastic component was most important from 50-80°C. At low temperatures hydrophobic interactions which help to maintain the gel structure are weakened, allowing the gels to be deformed. At higher temperatures (40-80°C), the hydrophobic interactions become stronger causing the gels to become more elastic.

Gill and Tung (1978) measured the steady shear flow behavior of gels made from the 12S glycoprotein of rapeseed (canola) using a Weissenberg Rheogoniometer. They also measured the viscoelastic response of the various canola gels by evaluating the dynamic shear stress response to small amplitude oscillation. The viscoelastic

response was measured in terms of the loss tangents (G"/G') as a function of oscillatory frequency . At pH 2, the gels had very high tangent values and low values at pHs of 8.2 and 10. Gels formed at pH 6.0 had elastic behavior similar to the gels formed at higher pHs. Measurement of the apparent viscosities of the different gel solutions indicated that the strongest gels tended to be formed at high pH and ionic strength. Aging of the dispersions at 4°C before gelation tended to increase the apparent viscosity of the solutions.

E. Dry Bean Flour and High Protein Products in Food Systems

Legume flours have been tested in a variety of food systems, most of them baked products.

1. Legume Flours and High Protein Products

Thompson (1977) investigated the use of mung bean isolate prepared by isoelectric precipitation as a protein supplement in bread . Breads with the isolate at the 10% substitution level were acceptable and had 41% more protein and a 73% higher PER than the unsupplemented bread.

Fleming and Sosulski (1977) investigated the nutritive value of bread fortified with plant protein concentrates from fababeans and field peas. Concentrates

were produced by pin-milling and air classification. Fababean and field pea bread gave PER's ranging from 1.7 to 1.8 and were given an "excellent" protein rating of more than 40. The bread diets were limiting in lysine and its addition caused a marked increase in protein quality.

Nielsen et al. (1980) prepared noodles and spaghetti from wheat flour fortified with 33% pea flour or 20% air-classified pea protein concentrate. Fortification increased the protein content to 22% for the noodles and 24% for the spaghetti. The fortifications reduced the cooking time for the noodles but cooking losses were higher. Addition of the pea products to spaghetti resulted in a product which had a texture similar to durum spaghetti but of inferior flavor and low tolerance to overcooking.

Patel et al. (1980) investigated the use of texturized navy bean protein concentrate (TNPC) as a meat extender in frankfurters both with and without non-fat dry milk (NFDM). The TNPC was produced by the air-classification of navy bean flour followed by drying a 35% aqueous slurry of the concentrate on a drum dryer. In one set of samples (TNPC/NFDM), TNPC was substituted for 10-30% of the beef while in the other TNPC it was substituted for 10-30% of both the NFDM and the beef. The TNPC frankfurters had higher processing shrinkage, softer textures, and more moisture and fat than the frankfurter containing TNPC/NFDM or the control. TNPC affected color,

texture, and flavor characteristics of the frankfurters to a greater degree than juiciness or greasiness. Neither of the frankfurters made with TNPC added were as well liked as the control. It was concluded that TNPC was acceptable only at low substitution levels.

Bread-baking properties of protein isolates prepared by Hsu et al. (1982) from germinated and ungerminated fababeans generally were better than those from peas or lentils. Germination had an adverse effect on the baking properties of protein isolates from fababeans, but not on those from peas or lentils.

Bahnassey et al. (1986) prepared flour by dry milling navy, pinto beans, and lentils and also prepared protein concentrates from the legume flours using the alkali extraction procedure of Fan and Sosulski (1974). The legume products have significantly higher protein, ash, fiber, and fat contents than the durum wheat semolina. A fortified spaghetti was prepared from blends of legume flour or protein concentrates with a strong gluten durum semolina. The protein, ash, and fiber contents of the fortified spaghetti prepared by Bahnassey et al. (1986) were higher than the control spaghetti. The amino acid composition of spaghetti made with the legume products showed a better balance of lysine and sulfur amino acids than that made solely from wheat. The legume flours and concentrates had a relatively higher level of most amino acids than the durum semolina.

Bahnassey and Khan (1986) investigated the rheological, processing, and quality of spaghetti fortified with legume flours or concentrates made from these flours. The legume products were prepared as described by Bahnassey et al. (1986). Spaghetti was prepared from durum wheat semolina blended with 3% vital wheat gluten and fortified with 0.5,10,15,20,and 25% legume flour or protein concentrate. The supplemented spaghetti showed an increase in farinograph water absorption except at the 25% level which had a slight decrease. The supplemented spaghetti also showed a decrease in the mechanical tolerance index and shattered earlier than the control. Supplemented spaghetti was shown by Bahnassey and Khan (1986) to decrease in cooked weight and increase in cooking loss as the level of fortification was increased. Spaghetti supplemented with up to 10% legume flours or protein concentrates was judged acceptable by a taste panel for all tested parameters; however, the panel tended to prefer spaghetti containing legume flours over the spaghetti containing concentrates. A beany taste was reported for spaghetti containing 25% legume flours or concentrates.

Satterlee et al. (1975) studied the use of a salt-extracted type protein concentrate in food systems. When the concentrate was added to bread, a decrease in the loaf volume occurred which corresponded to an increase in protein content. The bean proteins when added to sugar

cookies enhanced the width:height ratio.

2. Food Systems Applications of Navy and Pinto Bean Products

The potential applications for bean flour and air classified flour fractions produced by the method of Aguilera et al. (1982a and 1982b) in food systems were studied. The results of these investigations into the applications for bean products in food systems have been summarized by Uebersax and Zabik (1988).

a. Applications of Navy Bean Flours

The whole navy bean flour was tested for its effects in quick breads (Dwyer et al., 1982). A high quality pumpkin bread was produced with navy bean flour substituted for 35% of the wheat flour. The resulting bread contained 25% more protein than the control.

Whole navy bean flour was also used in apple spice cake, yeast breads, and as a substitute for wheat flour in bread (Uebersax et al. 1982) Rheological data, baking properties, and sensory evaluations indicated the navy bean flour was a good supplement for wheat flour in baking. The flour was used to replace up to 45% of the wheat flour in highly flavored baked goods without adversely affecting physical or sensory characteristics. However, yeast bread color, volume, and flavor were adversely affected by 20% substitution of navy bean flour for bread flour.

Navy bean flour was also substituted for up to 50% of the wheat flour in pumpkin bread, with 35% being the optimum substitution level (Uebersax et al., 1980). The presence of bean flour was found to increase the protein quantity and quality of pumpkin bread.

Bread flour was substituted at 0, 5, 15, and 30% with bean flour and incorporated into Chinese steamed bread (Zabik and Uebersax, 1986). Increased substitution with bean flour decreased volume while tenderness increased slightly.

The navy hull fraction was tested for its ability to increase the fiber content of sugar snap cookies (DeFouw et al., 1982b) and spice flavored layer cakes (DeFouw et al., 1982a), banana bread and in wheat bread (Uebersax et al., 1982). The high starch navy flour was evaluated in bean flour puffs and was used to formulate refried beans (Zabik and Uebersax, 1986).

The high protein navy fraction was tested for its potential as an additive in comminuted meat products (Uebersax et al., 1982), by incorporating the fraction into frankfurters at different protein levels. Frankfurters with 3.9% protein flour yielded an acceptable product. Frankfurters substituted at 7.9% were marginally acceptable, while substitution at the 11.9% level produced an unacceptable product.

High protein flour was also incorporated into banana bread and doughnut holes (Uebersax et al., 1982). High

quality banana bread with 0, 15, and 30% high protein flour was produced. This bread was as acceptable as the control to a taste panel. A high quality raised doughnut hole could be produced by substituting 25% of the wheat flour with high protein navy flour. It was suggested that the flour could help improve the nutritional quality of snack foods since the protein flour has four times the protein of wheat flour and is high in lysine.

High protein flours from navy, pinto, and black beans were substituted for all-purpose flour in cake doughnuts at levels of 0-30% (Spink et al., 1984). Doughnuts with 30% navy or pinto flour spread less than the control during cooking, while navy bean protein substituted at a level of 10 and 20% produced more tender doughnuts than those made from the black beans or the control. Doughnuts with a 13% substitution of navy or pinto protein were judged most acceptable by a consumer panel and also had less fat, were softer, and showed less firming after six days storage.

b. Application of Pinto Bean Flours

Whole pinto bean cotyledon flour was used as a substitute for wheat flour in a "master mix" to prepare muffins and pancakes (Zabik and Uebersax, 1986). Muffins substituted at the 20% level were slightly less acceptable than those made from control flour. Pancakes with 20%

substitution scored higher than the controls in all categories except flavor.

A standard egg noodle recipe was prepared with 0, 5, 10, and 20% of the standard wheat flour replaced with pinto flour (Zabik and Uebersax, 1986). Noodles with bean flour were more tender and slightly darker. Sensory evaluation indicated that increased substitution led to decreased flavor and acceptability. When the high protein bean flour was substituted for wheat flour in doughnuts, the final product was redder and the flavor attributes higher than the control. In general, substitution of 20-30% of the flour were feasible in all three food systems (Zabik et al., 1987).

Breads were also prepared using blends of wheat and pinto flour and bread flour and pinto flour. The breads baked with the wheat and 10% pinto flour had a slight decrease in volume, were more tender, and had excellent sensory characteristics compared to the bread and pinto flour mixture. Breads with the bread and pinto flour blend had fewer differences in volume and tenderness than the wheat/pinto flour blend (Zabik et al., 1987).

F. Effects of Processing on Digestibility of Dry Bean Proteins

1. Effects of Alkali Processing on Nutritional Quality

The most common method for producing protein isolates and concentrates from plant proteins is by solubilization of the proteins at high (alkaline) pHs followed by

precipitation of the protein at the isoelectric point. This method has the advantage of isolating protein in relatively high amounts using a simple procedure. Other processing methods employ high-pH processing such as texturization of proteins for the production of meat analogs, etc.

Alkali processing can produce numerous deleterious chemical reactions when proteins are exposed to high phs for long periods of time. Whitaker and Feeney (1983) reviewed and described the general deleterious protein reactions which include: hydrolysis, β-elimination, racemization, and crosslinking.

Deamidation is an important reaction in plant proteins. It can occur under relatively mild conditions: complete deamidation of glutamine residues may occur at pH 6.5 and 100°C, while the deamidation of asparagine occurs at pH 9 or 10 (Vickery et al., 1935). Plant proteins contain large numbers of asparagine and glutamine residues; however, little information is available on the extent of deamidation and its effect on the solubility and functionality of the proteins (Whitaker and Feeney, 1983).

β-elimination involves cystine, cysteine, serine, and threonine residues. The rate of loss of these amino acids is dependent upon the specific protein involved. Nashef et al. (1977) found that alkali treatment of different disulfide-containing proteins resulted in the formation of similar products although the formation reactions had

different energies of activation. The main formation reaction appeared to be β-elimination of the disulfides resulting in the formation of dehydroalanine. The authors proposed mechanisms in which dehydroalanine was a precursor in the formation of lysinoalanine, lanthionine, and β-aminoalanine. Other factors which affect the loss of sulfur amino acids are: pH, temperature, type of cations, and ionic strength (Whitaker and Feeney, 1983).

Two reactions which have received the greatest attention are racemization of amino acids in and crosslinking between alanine derivatives and lysine to form lysinoalanine (LAL). Using alfalfa leaf protein concentrate (LPC), lactalbumin (LA), and soy protein isolate (SPI), Schwass and Finley (1984) investigated racemization and lysinoalanine formation as influenced by time, temperature, and pH. Temperature had a greater effect on racemization than protein variety or time of reaction in 0.1N NaOH. Serine and aspartic acid/asparagine had the greatest amount of racemization in 0.1N NaOH.

Serine was found to be the amino acid most sensitive to pH and subsequent formation of racemers. Racemization of serine began at pH 7.0 and was significant at pH 8.5 for SPI and LPC but not for LA. At pH 10.0, the differences between SPI, LPC, and LA in serine racemer content were still apparent but not as great, whereas at pH 12.8 racemization of the serine in all three protein

isolates was complete. The authors speculated that these differences were caused by differences in denaturation between the proteins. Aspartate/asparagine was found to be the most stable with racemization beginning at pH 10 and continuing until pH 12.8. Again, LA had the least racemization. No significant racemization of the aspartate/asparagine occurred below pH 10.0.

LAL formation began at pH 8.5 for all proteins and then leveled off at pH 10 for SPI and LPC, while LA showed increased LAL formation up to pH 12.8. The study also showed LAL formation started above 55°C and began decreasing at 100°C.

Liardon and Hurrell (1983) studied the racemization of amino acids in casein heated at 80°C in 1M NaOH. Their procedure involved labeling D-isomers formed during the alkali treatment with deuterium and then using gas chromatography to separate the two isomers. Under these conditions, ASP, SER, TYR, and HIS were completely racemized while ALA, MET, PHE, LYS, AND GLU were more than 40% racemized. The authors concluded that alkaline solubilization is the most effective method for producing D-enantiomers.

Friedman et al. (1985) investigated some of the possible reactions between LAL in alkali treated proteins and Carboxypeptidase A (CPA). They demonstrated that LAL could inhibit metalloenzymes; however, free LAL and LAL bound in polypeptide chains appeared to inhibit by

different mechanisms. Free LAL appears to remove the zinc co-factor from the active site of CPA, while bound LAL acts on the CPA by either competitive inhibition from a molecule similar in shape to the substrate, masking of the active site through non-competitive inhibition, or both types of inhibition may be present. Inhibition of CPA by alkali treated proteins was thought to be important by Friedman et al. (1985) during protein digestion because it might produce effects similar to naturally occurring carboxypeptidase and soybean trypsin inhibitors.

Steinberg et al. (1975) examined the extent of LAL formation in hydrolyzed vegetable protein and soy protein isolate. These two products contained LAL in relatively low amounts: 40-500 and 0-370 μg LAL/g protein, respectively. They also found that LAL may form in proteins even though the proteins haven't been exposed to alkali treatment.

After reviewing the literature, Finot (1983) concluded that LAL caused only a slight reduction in nutritive value in conventional foods. The products which were high in LAL were those which had been subjected to severe alkaline treatments.

Nashef et al. (1977) investigated the effects of alkali treatment on different proteins. Lysozyme and several proteins were treated in 0.1 M NaOH at 50°C for 24 hr. This treatment resulted in the loss of cystine and lysine and in the formation of lanthionine, LAL, and

β-aminoalanine. These compounds were apparently formed by the β-elimination of disulfides to form the intermediate, dehydroalanine. Nucleophilic addition of various groups to the intermediate then led to the formation of the final amino acid products.

Much research has been done to construct a system which can measure the "digestibility" of proteins subjected to different processing conditions such as high pH or heat. Traditionally, determinations of this type have been performed using rat assays. However, these are both expensive and time consuming and much research has been conducted to develop *in vitro* digestibility systems.

2. Methods of Studying Digestibility of Proteins

The traditional methods for measuring the quality of proteins in food systems have usually involved the use of test animals. These methods have all been varients of the PER technique in which the quality and digestibility of the protein is based upon the growth of weanling rats when the protein in question is the sole source of nitrogen. In recent years, various authors have attempted to develop *in vitro* digestibility systems using various digestive enzymes to estimate protein quality.

Several methods for estimating protein digestibility involved the use of two enzyme systems in which the digestions were performed by the enzymes in sequence. For example, Akeson and Stahmann (1964) developed a two-step

digestion system using pepsin followed by pancreatin. The amino acids released by the digestion were quantitated by amino acid analysis which allowed calculation of a pepsin/pancreatin index with whole egg being used as a standard. The indices obtained from various proteins correlated well with literature values.

Saunders et al. (1972) measured protein digestibility using either pepsin/trypsin or pepsin/pancreatin. These two systems had a high degree of correlation with digestiblity values obtained in rat feeding trials.

Vachon et al. (1982) used a pepsin/pancreatin enzyme mixture to perform a two-step digestion to measure protein digestibilty. The pepsin digestion was performed at pH 2.0 for 30 min. The pH of the protein solution was then raised to 8.0 and the proteins were further digested for 24 hrs with pancreatin. The digestion was performed in a dialysis bag which imitated absorption in the intestine and prevented inhibition of proteolysis. The apparatus for performing this procedure was later improved (Gauthier et al., 1982; Savoie and Gauthier, 1986).

The C-PER (Calculated Protein Efficiency Ratio) was developed by Satterlee et al. (1982) as an alternative to rat bioassays for routine quality control screening of foods and food ingredients for protein quality. The C-PER is determined by using the amino acid profile and the apparent digestibility in a 4-enzyme digestion procedure.

Hsu et al. (1977) developed a multi-enzyme procedure composed of trypsin, chymotrypsin, and peptidase for the estimation of protein digestibility. In this system, a protein suspension was digested for 10 min followed immediately by a measurement of the pH of the solution. Results from this technique were highly correlated with *in vivo* protein digestibility as determined using rats. The authors believed that the biggest advantage of this system was the speed and the sensitivity of the measurement.

Porter et al. (1984) developed an immobilized enzyme system to determine the digestibility of food proteins. The system was composed of two columns, one containing pepsin and the other trypsin, chymotrypsin, and intestinal mucosal peptidases. Porter et al. (1984) found that the technique gave good agreement with in vivo digestibilities from the literature and was relatively stable under repeated use. The authors later found that this system was very sensitive to protein modifications which resulted from alkali treatment or Maillard reactions (Chung et al., 1986).

III. RATIONALE AND OBJECTIVES

A. Rationale for Dissertation Research

This investigation was designed to investigate the properties of high protein products from dry beans which may enable them to be used as food ingredients. Per capita consumption of dry beans has decreased drastically over the past several decades. This has led to interest in the development of food ingredients from dry beans which would be similar to those derived from soy proteins. In order to develop food ingredients from dry beans, more information must be obtained about the protein material itself and how it may function in food systems. The studies in this dissertation were all designed to provide this type of information.

B. Objectives of Study I: Evaluation of Methods for Production of High Protein Flours and Concentrates from Dry Beans (Phaseolus vulgaris)

This study evaluated different methods for processing of beans into high protein flours and isoelectrically precipitated concentrates. These materials could provide new opportunities for development of new bean ingredients for fabricated foods (Aguilera et al., 1981). Developing bean ingredients which can be used in the fabrication of foods is important because "fabricated engineered foods will increase, with raw materials coming from unconventional sources within plants, animals, and

microorganisms. From a technical standpoint there is a vast universe of materials that we (the food industry) can produce from proteins" (Lund, 1986). Two potential ingredients from dry beans are high protein flours and protein concentrates. In the first part of this investigation, an air classification scheme for the production of high protein flour was evaluated in terms of the protein content of the flour and a materials flow for the product. In the second part of this investigation, several methods for the production of protein concentrates were evaluated in terms of their yield and of the protein content of the material involved. Most of these methods involved isoelectric precipitation of the proteins, several used high salt concentration to isolate the proteins, and one involved solvent extraction of whole navy beans. The percent protein in the extracts was determined.

C. Objectives of Study II. Determination of the Functional Properties and Effects of Processing on High Protein Flours and Isoelectric Concentrates of Dry Beans (Phaseolus vulgaris)

This study had two main objectives:1. to determine the effects of two processing methods on the composition of the resulting products in comparison to the meal from which it was derived, and, 2. to compare the functionality of the high protein products with those of similar soy protein products.

Gel strength was determined on the 10% protein solutions using the back extrusion method of Harper et al. (1978) and Hickson et al. (1982). Back extrusion was performed on an Instron Universal Testing Machine (Model 4202, Canton, MA) equipped with a 50 N load cell using a crosshead speed of 100 mm/min. "Gel strength" or work required to penetrate the gel was calculated as the area under the force deformation curve.

Possible changes in the protein molecules brought about by the processing were determined by SDS-PAGE and by the soluble protein content of the high protein flour and the concentrate from each bean. The nutritional quality of the bean products was estimated by determining protein digestibility and methionine content. Some functional properties of the high protein flours, the isoelectric precipitates, and comparable soy products were determined. The functional properties investigated included: foaming capacity and stability, emulsification ability, gel strength, protein solubility at pH 6.5.

D. Objectives of Study III. Effects of pH and NaCl Concentration on Progel Formation of a Navy Bean Concentrate

In this study, progel formation of a navy protein concentrate (NPC) produced by isoelectric precipitation was evaluated at several pHs and NaCl concentrations. Progel formation characteristics were determined using dynamic rheological techniques. Results were compared to

changes in ANS hydrophobicity and protein solubility which occurred as navy protein concentrate solutions were heated at the pH and NaCl levels used previously.

IV. MATERIALS AND METHODS

A. Sources of Dry Beans Used

The materials used throughout the three investigations were derived from Michigan navy and kidney beans (*Phaseolus vulgaris*) purchased from a local supplier. The high protein soy materials used in Study II were Soyafluff 200W and Promax 70L, donated by Central Soya (Ft. Wayne, IN). Soyafluff is described by the manufacturer as a soy flour which is a "dry, mechanically classified product obtained from defatted and dehulled soybeans". Promax is described by the manufacturer as "a powdered, free flowing, uncolored, soy protein concentrate." The manufacturer would not give specific details, but it was presumed that the flour was made by air classification while the soy concentrate was made by hot water or alcohol extraction.

B. Dehulling of the Dry Beans

Before bean flours were produced in any of the studies, the dry beans were dehulled to remove much of the nondigestible material usually associated with beans. The beans were dried in a tray dryer at 63°C for 2 hr to improve their cracking ability and to aid in the removal of the hulls. The beans were next dehulled by passing

them through a hammermill using a screen with 3.8 cm. (1.5 in.) diameter holes. This allowed the bean hulls to be cracked and separated from the beans without causing any substantial size reduction in the beans themselves. By visual inspection it was estimated that approximately 70% of the hulls were removed. The hulls were separated from the beans by placing a tray containing a mixture of beans and hulls under a Plexiglas dome from an air-classifier and directing an air stream from a shop vacuum into the dome. The hulls were blown out from the tray and settled onto a piece of plastic which was resting on the floor underneath the tray. The separated hulls were periodically removed from the dome and discarded. This process was continued until most of the major hull fragments had been removed.

C. Production of High Protein Flours (Study 2)

The high-protein flours were produced according to the flow diagram shown in Figure 1. The numbers referred to as screen size are the decimal equivalents of the diameters of the holes in the screen in fractions of an inch.

The process consisted of grinding the dehulled beans into a flour using a hammermill then separating the flour into a coarse and fine fraction using a pilot-plant size air classifier. The air flow rate in the air classifier

was 3.5 m^3/hr which was the air-flow at which the bobber in the air flow indicator was halfway up the air indicator column. The fine fraction was saved while the coarse fraction was re-ground using a smaller screen size and the resulting flour was re-separated into a coarse and fine fraction using the air classifier. When the procedure was used for the production of a large quantity of flour, the fine fraction from each grinding/milling step was collected and combined with fine fractions from the other processing steps.

D. Production of Dry Bean Protein Concentrates

1. Evaluation of Methods for Producing Protein Concentrates (Study 1.)

Several methods for producing concentrates from navy bean flour were assessed. These methods are given in detail below:

METHOD

Fan and Sosulski (1974): Bean flour extracted 1 hr in 0.02% NaOH; Centrifuge 15 min at 1500 x g; Extract residue 1 hr in 0.02% NaOH. Centrifuge; Combine supernatents; Adjust pH to 4.5 using conc.HCl; Centrifuge; Residue washed and freeze-dried.

Alkaline Extraction (Modification #1): Performed using Fan and Sosulski except first extraction performed for 24 hrs.

Alkaline extraction (Modification #2): Same as Fan and Sosulski except 20,000 x g centrifugation used.

Alkaline extraction (Modification #3): Same as Fan and Sosulski except first extraction performed for 3 hrs and 20,000 x g centrifugation used.

Alkaline extraction (Modification #4): Performed using Fan and Sosulski except 15,000 x g centrifugation used.

Defatting of Navy Bean Flour: Bean flour was defatted using Goldfisch extraction and was then extracted using procedure of Fan and Sosulski.

Acetone Extraction: Protein extracted using Fan and Sosulski. Precipitated proteins were re-suspended in 0.02% NaOH and then precipitated using cold acetone.

Salting out: Extract 15g flour in 100 ml 1.7% NaCl for 30 min at 37°C; Centrifuge 5000 x g for 10 min; Dilute supernatent in tap water to precipitate; Centrifuge; freeze dry pellet.

Salting out (Modification #1): Same as above except pH of supernatent lowered to 4.5 using conc. HCl.

Starch Removal from Bean Flour: Proteins extracted using Fan and Sosulski (1974). Proteins then resuspended in 0.1M acetate buffer at pH 4.5 containing 20mM $CaCl_2$. Amyloglucosidase was added and starch digested overnight at 55°C. Following digestion, pH was lowered to 4.5 and solution centrifuged at 15,000 x g. Resulting pellet was resuspended in distilled H_2O and the pH reduced to 4.5 again.

Ma and Bliss Salt Extraction: Bean flour extracted using salting out procedure of Ma and Bliss (1978) which isolated vicilin fraction.

Salt extraction: Performed using Ma and Bliss except high protein navy flour (48% protein) used as starting material.

Sathe and Salunkhe (1981b) Extraction: Extract proteins using 0.5% Na_2CO_3. Centrifuge 10,000 x g for 30 min; Dialyze supernatent 48 hr at 4°C; Centrifuge again. Freeze dry pellet.

Whole Navy Bean Extraction: Whole beans soaked 12 hr in water. Beans ground in Waring Blender for 2 min. Beans placed into 1:5 solution of 0.02% NaOH: beans and ground in Waring Blendor. Proteins extracted at pH 8.5 for 3 hr. Solution centrifuged at 20,000 x g for 30 min; Supernatent was filtered through glass wool and #52 Whatman filter paper. Proteins precipitated at pH 4.5 using 0.05 N HCl. Precipitation was designed to be complete in 15-30 min. Solution was centrifuged for 30 min at 20,000 x g.

2. Isoelectrically Precipitated Bean Products (Study II)

The method of Fan and Sosulski (1974) was used with slight modifications. The protein concentrates were made in small batches which were kept frozen until enough had accumulated to produce a large quantity of precipitated material. The authors were not specific about the ratio of flour to water which was used in their procedure. A ratio of 5:1 was chosen because it made a very liquid solution but yet was small enough that it could be conveniently centrifuged.

The samples were freeze dried (Model 42; Virtis Co., Gardiner, NY) for three days using a chamber vacuum of 0.02 mm. The drier shelf temperature started out at 45.5°C and was allowed to rise to about 13°C. The dried material was put in sealable plastic bags and stored desiccated at room temperature.

The bean flours used as the starting materials for this procedure were ground (without air classification) using the procedure listed in Figure 1 (Study I).

3. Navy Concentrate Production (Study III)

About 2000g of dehulled navy flour was extracted using the method shown in Figure 4 to produce the protein concentrate. This method is a modification of Fan and

Sosulski (1974) and was shown by the work conducted in Study I to give a high protein yield. The concentrate was stored in brown jars which were placed in a desiccator and kept in a freezer at -30°C.

E. Analytical Methods

1. Compositional Analyses

a. Protein

Protein content in Study I and for the meals and high protein flours in Study II was determined using AACC Method 46-13: Crude Protein by the Micro-Kjeldahl Method (AACC, 1983). In Studies 2 and 3, the protein content of the soy products and the dry bean concentrates was determined using AOAC Method 24.038-24.040 Crude Protein in Meat; Block Digestion Method (AOAC, 1984). The following modifications were made in the Block Digestion Method: a sample weight of 0.1 g was used, 5 ml instead of 15 ml of H_2SO_4 were used, and 0 ml H_2O_2 (instead of 3ml) were added to the sample. All protein determinations were run in triplicate and the protein content was determined on a dry weight basis using a nitrogen conversion factor of 6.25.

b. Moisture and Ash

Moisture was determined using AACC Method 44-40: Modified Vacuum Oven Method (AACC, 1983) and ash was determined using AACC Method 08-01: Total Ash (AACC, 1983). This method was slightly modified to obtain complete ashing of the high protein materials. The crucibles containing dried samples from the moisture determination were put on a hot plate under the hood. The hot plate was set on the highest temperature setting and concentrated HCl was added until the samples were completely saturated. The samples dried until a gray ash started to form on the top and were then put into the muffle oven overnight. Both the moisture and the ash content were determined in triplicate.

c. Lipid (Study II)

The amount of lipid in the bean meals, high protein flours, and the concentrates was determined using the AOAC Direct Ether Extraction Method (7.047-7.048, 14.018; AOAC, 1984). This determination was done in duplicate.

d. Starch

The starch content of the high protein dry bean and soy flours and the dry bean and soy concentrates in Study II was determined in triplicate using the method of

Tomkinson (1986). A 19-21 mg sample was weighed into a 50 ml centrifuge tube containing 1 ml of water. The tube was put into a boiling water bath for 10 min, cooled, and then 4.5 ml of 0.5N NaOH was added. The sample was homogenized for 2 min using a Tekmar Tissumizer Model SDT (Tekmar, Co., Cincinnati, OH). Following homogenization, 10 ml of 0.04M acetate buffer, 2mg/ml amyloglucosidase with an activity of 10,000 units/g solid (Sigma Chemical Co., St. Louis,MO) and several crystals of thymol were added. The sample was re-homogenized and adjusted to pH 4.5 using conc. HCl. A blank containing the acid, base, and enzyme solution was also prepared. The solution was incubated for 8 hr in a shaking water bath at 55°C and then frozen until it could by analyzed for glucose content in a YSI Model 27 Industrial Analyzer (Yellow Springs Instrument Co., Yellow Springs, OH) by the method of Budke (1984).

In Study III, the starch content of the concentrate was determined by weighing out 150 to 175 mg of the navy protein concentrate, adding 2 ml of dimethylsulfoxide, and heating the samples in a boiling water bath for 1 hr to solubilize the starch. The samples were cooled and 8 ml of 0.1M acetate buffer at pH 4.5 containing 20 mM $CaCl_2$ was added. This was mixed and 5 ml of 5mg/ml of amyloglucosidase in 0.1M acetate buffer at pH 4.5 containing 20 mM $CaCl_2$ was added and allowed to incubate for 24 hr at 42°C with frequent mixing. Following

incubation, the samples were filtered through cheesecloth and frozen until they could be analyzed for glucose content using the method of Budke (1984).

2. Electrophoresis of Bean Products (Studies II and III)

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the bean meal and high protein flours and concentrates in Study II and the navy bean concentrate and the soluble proteins of the concentrate in Study 3 was performed on 12% (0.25% bis) acrylamide running gels with a 4% stacking gels using the system of Laemmli (1970).

Electrophoresis was performed with a Hoeffer Vertical Electrophoresis unit (Model SE 600; Hoeffer Scientific Instruments, San Francisco, CA) using a constant voltage power supply (Heathkit Model 1P-17, Benton Harbor, MI). A 0.25% protein (w/v) solution of each bean product was made up in Study II and a 1% (w/v) solution of the flour, concentrate, and the soluble proteins of each was made up in Study III. In each study, 10 µl of protein solution were put on the stacking gel. A constant current of 30 mA was applied until the proteins migrated into the running gel and then the current was increased method to 60 mA until the bromophenol blue tracking dye reached the bottom of the running gel. The gels were removed and stained for 6 hours in 0.125%

Coomassie Blue in 50/10/40 (v/v/v) methanol/acetic acid/water. The gels were destained in 7/5/88 (v/v/v) acetic acid/methanol/water until clear.

Subunit molecular weights were estimated using a mixture of low molecular weight proteins (Dalton Mark VII-L; Sigma Chemical Corp., St. Louis, Mo). The mixture consisted of the following proteins (with the listed molecular weights): α-lactalbumin (14.2 kilodalton), soybean trypsin inhibitor (20.1 kd), trypsinogen (24 kd), carbonic anhydrase (29 kd), glyceraldehyde 3-phosphate dehydrogenase (36 kd), egg albumin (45 kd), bovine albumin (66 kd). The relative mobility (RM) of the protein standards was calculated using the formula:

$$RM = \frac{\text{Distance of Protein Migration (cm)}}{\text{Marker Dye Distance (cm)}}$$

and a plot of relative mobility vs molecular weight was constructed. The relative mobility of each protein subunit was calculated and the molecular weight was estimated from the standard curve. In Study III, the amount of protein present in each band on the gels was determined using a Shimadzu Dual Wavelength Thin-Layer Chromato Scanner (Model cs-930, Kyoto, Japan). The

protein bands were identified by their subunit molecular weights.

F. Nutritional Analyses (Study II)

The methionine content and the digestibility of the proteins in the bean flour, high protein bean flour, and the bean protein isoelectric precipitates were determined in triplicate. These were performed to assess the effects of processing on the proteins in the bean products.

1. Methionine Content

The methionine content was determined using the gas chromatography (GC) method of Finlayson and MacKenzie (1978) as modified by MacKenzie (1977). The primary reaction of this determination is the preferential reaction of the methionyl group with cyanogen bromide, leading to the formation of homoserine and methyl thiocyanate. The amount of methyl thiocyanate detected is directly related to the amount of methionine present in the sample.

2. Digestibility

The digestibility of the proteins was determined using AOAC Method 43.265: *In Vitro Digestibility Method for*

C-PER (AOAC, 1984). The enzymes used in this determination were: porcine pancreatic trypsin (Type IX), porcine intestinal peptidase (Grade I), bovine pancreatic α-chymotrypsin (Type II) and bacterial protease (Pronase E). All enzymes were obtained from the Sigma Chemical Co., St. Louis, MO. The following modification was made from the published procedure: the reference casein diet (High Nitrogen Casein; U.S. Biochemical Corp., Cleveland, OH) and the bean samples were solubilized in 10 mls of water and the pH was increased into the alkaline range. The samples were stirred for 1 hr at 37°C. This change made it easier to solubilize the bean samples and the casein.

G. Functional Properties

Emulsification capacity, gelation characteristics, foam forming ability, foam stability, and protein solubility of the dry bean and soybean high protein flours and dry bean and soybean concentrates were determined empirically. All determinations were run in triplicate.

1. Foaming and Emulsification Characteristics (Study II)

Foaming capacity and stability were determined in a Waring Blendor at room temperature on a 1% protein

solution in 0.2M phosphate buffer, pH 6.5 using the method of Sathe and Salunkhe (1981a).

The emulsification test was performed using the method of Webb et al. (1970) with slight modifications. Samples containing 10 mg/ml protein with 0.2M NaCl were prepared in 0.2M phosphate buffer at pH 6.5. Fifty milliliters of sample at 4°C were emulsified using a Tekmar Tissumizer SDT (Tekmar, Cincinnati, Ohio).

Soybean oil was purchased locally and cooled to 10°-15°C. The oil was delivered from a 50 ml side-arm buret at a rate of 2.0 ml/min and a multimeter (Model 3550, Triplett Corp., Bluffton, OH) was used to measure the electrical resistance of the solution. The speed of the homogenizer was increased periodically to insure uniform mixing as the viscosity of the emulsion increased. The endpoint of the test was the point at which the electrical resistance of the solution increased drastically and the solution also became "creamy". Results were expressed as ml oil/ mg protein.

2. Gelation Properties (Study II)

The minimum protein concentrations which formed stable gels from the various protein products were assessed using the method of Coffman and Garcia (1977). Solutions of 1,2,4,6,8,10, and 12% protein were prepared in 0.2M phosphate buffer at pH 6.5. Five milliliters of

each solution were placed into 15 x 105 mm screw top test tubes. The tubes were heated in a circulating water bath for 15 min at 90°C. They were then cooled at in an ice bath for 5 min. The lowest protein concentration which formed a stable gel was determined to be the one which did not slip in an inverted tube.

The gelation properties of the protein materials were determined on 10% protein solutions in 0.2M phosphate buffer at pH 6.5. Five milliliters of each solution was placed into 15 x 105 mm screw top test tubes. The tubes were heated in a circulating water bath for 15 min at 90°C. They were then cooled in an ice bath for 5 min. The strength of the gels formed was determined using the back extrusion method of Harper et al. (1978) and Hickson et al. (1982). The back extrusion was performed on an Instron Universal Testing Machine (Model 4202, Canton, MA) equipped with a 50 N load cell using a crosshead speed of 100 mm/min. A computer attached to the Instron collected the data and performed numerical calculations on it. "Gel strength" was calculated as the area under the force deformation curve formed by puncturing the gel with the probe.

3. Soluble Protein Content (Studies II and III)

The soluble protein content of both the high protein flours and the isoelectric precipitates in Study II at pH 6.5 and the soluble protein content of the concentrate and

navy bean flour in Study III at pH 7.0 was determined using the method of Morr et al. (1985) This procedure was performed a total of six times: 2 separate samples were made up and the soluble protein content of each sample was determined three times. This method determines the amount of protein which is soluble at a given pH in 0.1M NaCl.

H. Hydrophobicity and Soluble Protein Content of Navy Bean Concentrate (Study III)

1. Preparation of Samples for Protein Solubility and Hydrophobicity

Solutions of 10mg of protein/ml for the solubility test and 1 mg/ml for hydrophobicity test were prepared at pH 4.5, 6.0, 7.0, and 8.0 with 0.2M Nacl and also at 0.0, 0.2, 0.4, and 0.6M NaCl with a pH of 7.0. Ten milliliter aliquots were placed in 15 x 105 mm screw cap test tubes and the test tubes were placed in a test tube rack. This test tube rack was then placed in a water bath equipped with a programmable temperature controller. The programmer was set to go from an initial temperature of 30° up to 95°C at a rate of 1°C/min. Samples were removed from the water bath at 30, 50, 70, 80, 90, and 94°C.

2. Change in Hydrophobicity of Navy Protein Concentrate

The hydrophobicity of the proteins in heated solutions of the navy protein concentrate was determined by reacting with ANS (8-anilino-1-naphthalene sulfonic

acid). After heating, the protein solutions were diluted with 0.2M phosphate buffer to obtain protein concentrations of 0.6, 0.10, 0.05, and 0.01 mg/ml. A reference solution consisting of 0.1 mg/ml BSA in 0.1 M buffer at pH 7.0 and an ANS stock solution (8mM ANS in 0.1M phosphate buffer, pH 7.0) were also prepared.

The fluorescence intensity (FI) for each solution was measured on a Varian Spectrofluorometer (Model SF-330; Varian Assoc., Palo Alto, CA) at an excitation wavelength of 366 and an emission wavelength of 481. The FI was standardized by mixing 2 ml of methanol and 10 µl of the ANS stock solution in a cuvette, allowing it to react for 1 min, and adjusting the reading on the fluorometer to give an FI of "150".

The FI of each protein concentration was determined by adding 10 µl of the ANS stock to 2 ml of each dilution, mixing, allowing the reaction to occur for 1 min and then measuring FI. The net FI of each dilution was calculated by subtracting the FI of each solution without the ANS from that of the solution with the ANS. The net FI of a 2 ml aliquot of the BSA solution was determined periodically during the session to assure that the fluorometer was still functioning properly. The slope of FI vs protein concentration (mg/ml) as determined by linear regression was used as a measure of absolute protein hydrophobicity.

From this data, a plot of absolute hydrophobicity vs heating temperature was constructed.

A relative hydrophobicity value for the proteins at each temperature was calculated to make comparison between samples easier. The relative hydrophobicity was calculated by converting all of the absolute hydrophobicities within a treatment to decimal fractions of the absolute hydrophobicity at 94°C.

A preliminary investigation was conducted to determine the heat stability of the navy protein concentrate using the ANS procedure. For this experiment, 25 ml of a 12% protein solution were made up in 0.2M phosphate buffer at pH 7.0. Five ml aliquots of this solution were placed into screw-cap test tubes and heated in a boiling water bath (approximately 95°C) for lengths of time ranging from 0 min to 60 min. The samples were diluted and the FI of the samples at each heating time were determined as previously described.

3. Solubility of Navy Concentrate Proteins

Protein solutions (10 mg/ml) were centrifuged for 30 min at 20,000 x g and the supernatent was filtered through Whatman #1 filter paper. Two milliliter aliquots of the filtered supernatent were placed in Kjeldahl tubes and the protein content was determined on a Brinkmann-Buchi Semi-Automated Kjeldahl unit using AOAC Method 24.038-24.040;

Crude Protein in Meat; Block Digestion Method (AOAC, 1984). The total protein content of each solution was also determined. Each sample was run in triplicate. The soluble protein content of each sample was determined by calculating the amount of protein in the supernatent and dividing it by the total amount of protein present in each sample. The total amount of protein present was found to very close to the theoretical amount of 10 mg/ml so this figure was used in the actual calculations. The amount of insoluble protein in each sample was calculated by subtracting the amount of soluble protein from 100.

I. Progel Formation of Navy Bean Concentrate (Study III)

1. Least Protein Content Needed for Gelation

The least amount of protein from the protein concentrate which was needed to form a gel was determined using the method of Sathe and Salunkhe (1981a) with slight modifications. Protein solutions ranging from 2 to 20% protein were prepared in 0.2M phosphate buffer at pH 7.0. Screw cap test tubes (15 x 105 mm) containing 5 ml of protein solution were placed in a constant 90°C water bath for 20 min. After heating, the gels were removed from the bath and placed into an ice bath for 10 min. They were then allowed to cool for 2 hr at 4°C before being tested. The least protein concentration necessary for gelation was determined to be the concentration yielding a structure

which did not slip down the tube when completely inverted. This procedure was conducted in duplicate.

2. Dynamic Rheological Properties of Navy Bean Concentrate

A Rheometrics Fluid Spectrometer (RFS) Model 8400 (Rheometrics Inc., Piscataway,NJ) was used to determine the dynamic rheological properties of the navy protein concentrate. A 120 mg/ml protein solution was made up at the desired pH and NaCl concentration and held at 4°C overnight. Before dynamic testing, the pH of the sample was re-checked and adjusted if necessary. For the test itself, 3 ml of sample were placed into the sample cup of a Rheometrics Fluid Spectrometer. The machine was set to perform dynamic testing using a cone and plate arrangement. The cone was lowered into the sample to a preset height of 35 µm above the plate. A constant temperature water bath with a temperature programmer attached to it was used to heat the sample at a constant rate of 1°C/min from 50 to 90°C. Ethylene glycol was used as the heating medium because of its relatively low volatility at high temperatures. All of the dynamic tests were started at 50°C because the results of the hydrophobicity testing and the solubility testing indicated that no transitions occurred before that temperature. The sample was placed in the 50°C bath and allowed to equilibrate for 5 min. Following

equilibration, the sample was heated and the G′ (storage modulus) and G" (loss modulus) of the sample recorded against time until there was no change in the sample's rheological properties. Generally, the samples reached a point at which there was no further increase in either loss or storage modulus. The tests were conducted until two reasonably consistent results were obtained from the same sample. From these data, two other factors were calculated: G* (complex modulus), a factor which is proportional to the total force needed to deform the sample, and tan δ (loss tangent) a factor numerically equal to G"/G′ which defines the ratio of liquid to solid characteristics of the material.

a.Strain and Frequency Sweeps

The sample strain and the frequency of oscillation conditions under which the fluid spectrometer tests were to be run were determined before the actual testing began. Frequency sweeps were conducted at 50, 75 and 90°C at frequencies from 1 to 100 rad/sec at strains from 0.1-10%. Strain sweeps were conducted from 0.01 to 100% at a frequencies ranging from 1-15. An outline of the tests conducted is given in Table 8 (Study III).

J. Statistical Analysis (Study II)

The means of the nutritional and functional property analyses were compared using a one-way analysis of variance (ANOVA). The means of all of the dry bean and soy products in a table were tested as one block. When significant differences between means were discovered, mean separation was determined using Duncan's Multiple Range Test.

V. STUDY I: EVALUATION OF METHODS FOR PRODUCTION OF HIGH PROTEIN FLOURS AND CONCENTRATES FROM DRY BEANS (Phaseolus vulgaris)

A. Introduction

Air classification has been shown by a number of researchers to be an effective method for the production of high protein flours from a number of legumes (Kon et al., 1977; Patel et al., 1980; Sosulski and Sosulski, 1986). In this process, the protein content of the high protein flour can be increased by regrinding and reclassifying the coarse fraction obtained after the first pass. In order to better understand this process and how it functions to separate proteins, many researchers perform proximate analyses of the various fractions obtained by this multi-step process (Vose et al., 1976; Tyler et al., 1981; Aguilera et al., 1982).

Isoelectric precipitation has long been used as a method for isolating the proteins of soybeans (Meyer, 1971; Kolar et al., 1985). Many researchers have also used this method for the production of concentrates from different legume species as a means of increasing the use of these materials in foods (Fan and Sosulski, 1974; Thompson, 1977; Musakhanian and Alli, 1987) Other methods for extracting proteins from legumes such as salting out have also been proposed by different researchers (Ma and Bliss, 1978; Murray et al., 1981).

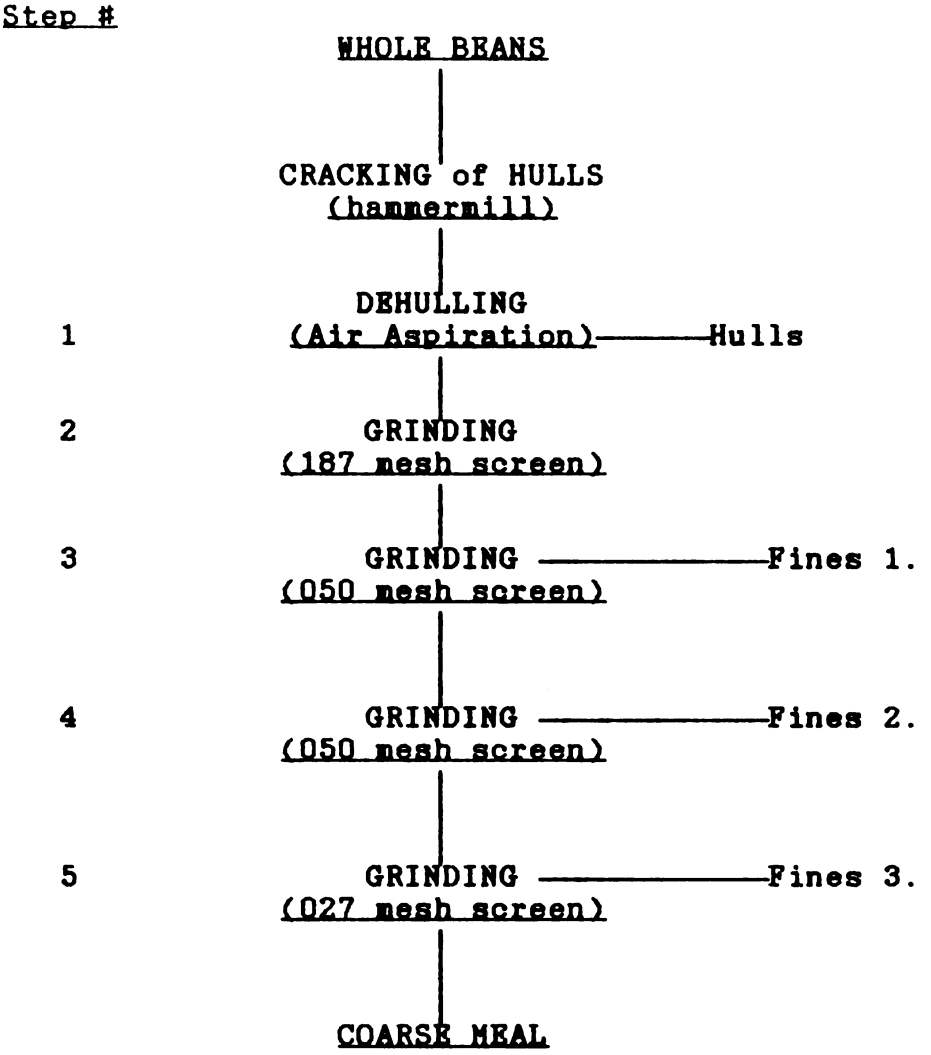

Figure 1. Process flow for production of high protein flours

A process for air classification of navy and kidney flours was developed for the production of high protein flours. The suitability of this procedure was assessed by determining the material balance of the process and the composition of the protein fraction produced. In the second part of the investigation, different methods were evaluated for the production of an isoelectric precipitate with a protein content of 85-90%.

B. Materials and Methods

The materials used for this investigation were derived from Michigan navy and kidney beans (*Phaseolus vulgaris*) which were purchased from a local supplier.

1. Air Classification

An overview of the air classification procedure is shown in Figure 1. Moisture was determined using AACC Method 44-40: Modified Vacuum Oven Method (AACC, 1983), ash was determined using AACC Method 08-01: Total Ash (AACC, 1983), and protein was determined using AACC Method 46-13: Crude Protein by the Micro-Kjeldahl Method (AACC, 1983) with a nitrogen conversion factor of 6.25.

2. Concentrate Production

The various methods which were evaluated for the production of concentrates from dry bean flour are outlined below:

1. Fan and Sosulski (1974): Bean flour extracted 1 hr in 0.02% NaOH; Centrifuge 15 min at 1500 x g; Extract residue 1 hr in 0.02% NaOH. Centrifuge; Combine supernatents; Adjust pH to 4.5 using conc.HCl; Centrifuge; Residue washed and freeze-dried.

2. Alkaline Extraction: Performed using Fan and Sosulski except first extraction performed for 24 hrs.

3. Alkaline extraction: Same as Fan and Sosulski except 15,000 x g centrifugation used.

4. Alkaline extraction: Same as Fan and Sosulski except first extraction performed for 3 hrs and 15,000 x g centrifugation used.

5. Alkaline extraction: Performed using Fan and Sosulski except 20,000 x g centrifugation used.

6. Defatting of Navy Bean Flour: Bean flour was defatted using Goldfisch extraction and was then extracted using procedure of Fan and Sosulski.

7. Acetone Extraction: Protein extracted using Fan and Sosulski. Precipitated proteins were re-suspended in 0.02% NaOH and then precipitated using cold acetone.

8. Salting out: Put 15g flour into 100 ml. of 1.7% NaCl solution. Extract 30 min at 37°C; Centrifuge 5000 x g for 10 min; Dilute supernatent in tap water to precipitate; Centrifuge; Freeze dry pellet.

9. Salting out: Same as above except pH of supernatent lowered to 4.5 using conc. HCl.

10. Starch Removal from Bean Flour: Proteins extracted using Fan and Sosulski. Proteins then resuspended in 0.1M acetate buffer at pH 4.5 containing 20mM $CaCl_2$. Amyloglucosidase was added and starch digested overnight at 55°C. Following digestion, pH was lowered to 4.5 and solution centrifuged at 15,000 x g. Resulting pellet was resuspended in distilled H_2O and the pH reduced to 4.5 again.

11. Ma and Bliss Salt Extraction: Bean flour extracted using salting out procedure of Ma and Bliss (1978) which isolated vicilin fraction.

12. Salt extraction: Performed using Ma and Bliss except high protein navy flour (48% protein) used as starting material.

13. Salunkhe Extraction: Extract proteins using 0.5% Na_2CO_3. Centrifuge 10,000 x g for 30 min; Dialyze supernatent 48 hr at 4°C.;Centrifuge again. Freeze dry pellet.

14. Whole Navy Bean Extraction: Whole beans soaked for 12 hrs in ddH_2O. Beans ground in Waring Blender for 2 min. Beans placed into 1:5 solution of 0.02% NaOH: beans before blending. Proteins extracted at pH 8.5 for 3 hrs. Solution centrifuged at 20,000 x g for 30 min; Supernatent was filtered through glass wool and #52 Whatman filter paper. Proteins precipitated at pH 4.5 using 0.05 N HCl with precipitation timed to occur for 15-30 min. Solution was centrifuged for 30 min at 20,000 x g.

In each evaluation, 50g of flour was used as the starting material. The total process yield was calculated using the formula:

total process yield=

$$\frac{\text{weight high protein material (g)}}{\text{50g flour}} \times 100\%$$

C. Results and Discussion

1. High Protein Flours

The partial composition of the fine fraction collected in each step of the air classification, the coarse cotyledon fraction and the yield of this procedure is shown in Tables 1 and 2. Visual examination of the fine fraction showed that it had a very fine texture similar to that of talcum powder while the coarse fraction had much larger particles in it and tended to look and feel like sand.

Table 1. Mass balance of process and composition of fine fraction collected during production of high protein flour from navy beans[1]

Processing Step	Yield (g)	Protein (%)	Ash (%)	Moisture (%)
Whole Beans	1000			
1				
Hulls	63.3	12.65	7.65	7.16
Coarse Meal	872.0			
2				
Coarse Meal	852.5			
3				
Fines 1.	20.6	49.47	8.13	5.74
Coarse Meal	753.1			
4				
Fines 2.	9.2	47.34	7.66	6.49
Coarse Meal	673.9			
5				
Fines 3.	21.3	46.29	7.59	6.58
Coarse Meal	573.0	20.32	3.51	8.42
Total Yield of High Protein Flour			5.1%	

[1]Difference between fine fraction collected and coarse meal is unaccountable loss

Table 2. Mass balance of process and composition of fine fraction collected during production of high protein flour from kidney beans[1]

Processing Step	Yield (g)	Protein (%)	ash (%)	moisture (%)
Whole Beans	1000			
1				
Hulls	56.7	5.10	3.94	7.92
Coarse Meal	878.0			
2				
Coarse Meal	861.8			
3				
Fines 1.	26.2	55.18	7.12	6.87
Coarse Meal	731.5			
4				
Fines 2.	12.1	49.88	6.98	8.58
Coarse Meal	600.4			
5				
Fines 3.	16.6	51.41	6.08	8.77
Coarse Meal	469.8	23.60	6.71	6.45
Total Yield High Protein Flour		6.1%		

[1]Difference between fine fraction collected and coarse meal is unaccountable loss

The protein content of the fine fraction from each processing step stayed relatively constant throughout the process, although it did drop slightly in the latter steps. This is similar to the results seen by Tyler et al. (1981) and Aguilera et al., (1982b). These results indicate that the yield of high protein flour can be increased without affecting the protein content of the flour.

The process itself was not very efficient. One would expect a yield of about 20-25% if all the protein in the bean was being separated into the air classified fine fraction. Instead the highest yield obtained was about 15% when large batches of beans were ground up and used for the flour. This would not preclude the use of this process for the production of high-protein flours since it has other advantages: among them, it allows one to double the protein content of the bean flour without using expensive solvents and without the resulting waste streams.

From the data, it is obvious that the process is more efficient when larger quantities of beans are ground up and air classified. This is probably a result of the way in which material was lost during the processing procedure. During the milling portion of the process, a large amount of material can be lost from the feed-pan or the bottom of the machine. The number of particles lost

tended to be constant regardless of particle size. Thus when the particle size was large, the amount of material lost was correspondingly greater. Attempts were made to minimize these losses during the processing by placing a large plastic bag over the feed-pan and placing a catch-pan as close to the bottom of the mill as possible.

Losses during the air classification segment occurred mainly during the beginning of the procedure. The fine fraction tended to be coated onto the discharge port and certain other parts of the air classifier. Once these parts were coated, the rest of the fine fraction could be collected. The fine fraction which was coating the air classifier was scraped off as best as possible at the end of the processing. Because the losses during air classification tended to remain constant, the amount of material lost tended to decrease as the quantity of beans processed increased.

2. Production of a Navy Bean Protein Concentrate

The protein content and yield of each concentrate production method is summarized in Table 3. Most of the resulting precipitates have protein contents around 80-83% (N x 6.25) except for the acetone extracted material (Method 7) which has a protein content of around 90%. The acetone extracted material had two characteristics which made it unsuitable as a protein concentrate. First, when an attempt was made to make a large quantity of the

Table 3. Methods evaluated for production of navy bean protein concentrate.

METHOD	PROTEIN (%)	PROCESS YIELD (%)
1	81.7	14
2	83.1	10
3	83.9	16
4	84.0	16
5	83.1	18
6	82.5	18
7	90.7	10
8	no reaction	
9	70.7	7
10	78.4	8
11	70.5	6
12	82.4	22
13	62.7	<1
14	77.1	10

material, it was found that the protein content dropped to about 83%. This may mean that the higher protein content of the preliminary batch was simply a coincidence or that for some unexplained reason the scale-up of the process caused the protein content to decrease.

The second problem with this material was that it was extremely insoluble as determined using the solubility procedure of Morr et al. (1985). It appears that the acetone caused dehydration of the material which led in turn to the formation of material which had crystalline characteristics following freeze-drying.

It is interesting to note that simply increasing the centrifugation speed increased the protein content (Methods 1-5) probably by increasing separation efficiency between the insoluble material and the soluble proteins. These results indicate that the method which gave results closest to what was desired was the one in which the Fan and Sosulski procedure was followed but the centrifugation steps were carried out at 20,000 x g instead of 1500 x g. (Method 3). Ma and Bliss (1978) were able to isolate the vicilin fraction of the dry beans using a salting out procedure. When this procedure was used in this investigation, the protein content was only 70.5% when whole navy bean flour was used. When high protein navy flour was used as the starting material, the protein content was 82.4%. It is obvious that it helped to partially isolate the proteins before the extraction was

performed. It is not known why the both varients of the procedure did not yield a higher protein content.

It is interesting to see that the procedures which involved salting out of the proteins all gave both a lower protein content and a low yield. The one exception was the salting out procedure of Ma and Bliss (1978) in which high protein flour was used as the starting material. This method resulted in a protein content which was similar to the isoelectric precipitation procedures and also had a very high yield. It appears that Ma and Bliss method works well when one starts with partially purified material.

D. Conclusions

The results of these investigations indicate that the lab scale air classification procedure will yield a large amount of high protein flour by remilling and reclassifying. Most of the losses experienced during the process can be minimized by reducing the amount of material lost during the initial milling steps. The second milling and air classification step with 050 mesh screen could be eliminated since the amount of high protein flour derived from this step is relatively small compared to the other steps.

The most satisfactory method for production of large amounts of a protein concentrate from dry bean flour is a

modification of the isoelectric precipitation method of Fan and Sosulski (1974) in which the centrifugation speed is increased to 20,000 x g.

VI. STUDY II. DETERMINATION OF THE FUNCTIONAL PROPERTIES AND EFFECTS OF PROCESSING ON HIGH PROTEIN FLOURS AND ISOELECTRIC CONCENTRATES OF DRY BEANS (Phaseolus vulgaris)

A. Introduction

Over the past twenty five years, the per capita consumption of beans in the U.S. has declined considerably. This has led to interest in new products which can overcome some of the nutritional problems associated with beans and also provide new opportunities for development of new bean ingredients for fabricated foods (Aguilera et al., 1981). Two potential ingredients from dry beans are high protein flours and protein concentrates. These products could improve the nutritional value of foods and impart desired functional properties to various food systems in the same ways that soy proteins have been used.

The production of high protein flours from dry beans has been studied extensively. Air classification has been used for the production of high protein flour from navy beans (Patel et al., 1980; Aguilera et al., 1981), California small white beans (Kon et al., 1977), and pea, northern beans, chickpeas, lima beans, fababeans, field peas, mung beans, and lentils (Sosulski and Youngs, 1979). The process of milling followed by air classification to obtain high protein flours has been reviewed by Sosulski (1983).

The production of protein concentrates has also been studied by many researchers. Fan and Sosulski (1974) used an isoelectric precipitation method to produce isolates from nine different legume meals including dry beans. The authors produced isolates which ranged from around 92-93% protein for soybean, lupine, and fababean to 82-83% for pea bean, lentil, and chickpea.

Sosulski and McCurdy (1987) investigated the functionality of high protein flours, protein isolates, and protein fractions from field peas and fababeans. The protein fractions were prepared by air classification while the isolates were prepared by either alkali extraction followed by isoelectric precipitation for the field peas or acid extraction and isoelectric precipitation for the fababeans. The functional characteristics of these products were compared to similar soy products. All of the proteins in flour and protein fractions were highly soluble at acid pH with a narrow range of insolubility at pH 4-5. Water holding and oil absorption increased as protein content increased for all products while oil emulsification properties were uniformly high among all products.

Very little work has been done to compare high protein flours and concentrates from navy and kidney beans with each other and with similar soy products. In this investigation, the composition and relative functionality of the protein products produced by air classification and

isoelectric precipitation were investigated. The objectives were: 1. to determine the effects of two processing methods on the composition of the resulting products in comparison to the meal from which it was derived, and, 2. to compare the functionality of the high protein products with those of similar soy protein products.

B. Materials and Methods

1. Production of High Protein Flours

High protein bean flours were prepared from dehulled navy and kidney beans as described in Study I.

2. Isoelectrically Precipitated Concentrates

Navy and kidney bean concentrates were prepared using the method of Fan and Sosulski (1974). The precipitates were freeze dried (Model 42; Virtis Co., Gardiner, NY), put in sealable plastic bags and stored desiccated at room temperature.

3. Compositional Analyses

The protein content of the meals and high protein flours was determined using AACC Method 46-13 (AACC, 1983), the protein content of the soy products and the dry bean concentrates was determined using AOAC Method 24.038-

24.040 (AOAC, 1984). All protein determinations were run in triplicate and the protein content was determined on a dry weight basis using a nitrogen conversion factor of 6.25. Moisture was determined using AACC Method 44-40 (AACC, 1983), ash was determined using AACC Method 08-01 (AACC, 1983) and the lipid content of the bean products was determined using AOAC Method 7.047-7.048, 14.018 (AOAC, 1984). All three determinations were made in duplicate.

The starch content of the high protein dry bean and soy flours and the dry bean and soy concentrates was determined in duplicate using the method of Tomkinson (1986). Glucose content of the digest was measured in a YSI Model 27 Industrial Analyzer (Yellow Springs Instrument Co., Yellow Springs, OH) by the method of Budke (1984).

4. Electrophoresis

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the bean meals and high protein flours and concentrates was performed on 12% (0.25% bis) acrylamide running gels with a 4% stacking gels using the system of Laemmli (1970). Electrophoresis was performed with a Hoeffer Vertical Electrophoresis unit (Model SE 600; Hoeffer Scientific Instruments, San Francisco, CA) using a constant voltage power supply

(Heathkit Model 1P-17, Benton Harbor, MI). Ten µl of a 0.25% (w/v) protein solution were put on the stacking gel. A constant current of 30 mA was applied until the proteins migrated into the running gel and then the current was increased method to 60mA until the bromophenol blue tracking dye reached the bottom of the running gel. The gels were removed and stained for 6 hours in 0.125% Coomassie Blue in 50/10/40 (v/v/v) methanol/acetic acid/water. The gels were destained in 7/5/88 (v/v/v) acetic acid/methanol/water until clear.

The relative mobility (RM) of each protein subunit was calculated and the molecular weight (MW) was estimated from a standard curve of RM vs. MW prepared using a mixture of low molecular weight (14.2 -66 kilodaltons) proteins (Sigma Chemical Corp., St. Louis, MO).

5.Nutritional Analyses

The methionine content was determined using the gas chromatography method of Finlayson and MacKenzie (1976) as modified by MacKenzie (1977). The digestibility of the proteins was determined using AOAC Method 43.265 (AOAC, 1984) with one modification. The reference casein diet (High Nitrogen Casein; U.S. Biochemical Corp., Cleveland, OH) and the bean samples were solubilized in 10 ml of

water, adjusted to pH 8.0, stirred for 1 hr at 37°C prior to analysis.

6.Functional Properties

Foaming capacity and stability were determined in a Waring Blendor at room temperature on a 1% protein solution in 0.2M phosphate buffer at pH 6.5 using the method of Sathe and Salunkhe (1981a). Foaming capacity was defined by determining the specific volume and the volume increase of the protein solution where:

$$\text{specific volume (ml/g)} = \frac{\text{volume after whipping}}{\text{weight after whipping}}$$

$$\text{volume increase (\%)} = \frac{\text{vol. after whipping - vol. before whipping}}{\text{vol. before whipping}}$$

The emulsification test was performed using the method of Webb et al. (1970) with slight modifications. Samples containing 10 mg/ml protein were prepared in 0.2M NaCl, 0.2M phosphate buffer, pH 6.5. Fifty milliliters of sample at 4°C was emulsified using a Tekmar Tissumizer SDT (Tekmar, Cincinnati, Ohio). Soybean oil was purchased locally and cooled to 10°-15°C. The oil was delivered from a 50 ml side-arm buret at a rate of 2.0 ml/min and a multimeter (Model 3550, Triplett Corp., Bluffton, OH) was used to measure the electrical resistance of the solution. The speed of the homogenizer was increased periodically to insure uniform mixing as the viscosity of the emulsion increased. The endpoint of the test was the point at

which the electrical resistance of the solution experienced a large increase and the solution also became "creamy". Results were expressed as ml of oil per mg protein.

The minimum protein concentrations which formed stable gels from the various protein products were assessed using the method of Coffman and Garcia (1977) except that solutions of 1,2,4,6,8,10, and 12% protein were prepared in 0.2M phosphate buffer at pH 6.5. The gelation properties of the high protein materials were determined on 10% protein solutions in 0.2M phosphate buffer at pH 6.5. In both determinations, 5 ml of each solution was placed into 15 x 105 mm screw top test tubes. The tubes were heated in a circulating water bath for 15 min at 90°C and cooled at in an ice bath for 5 min.

Gel strength was determined on the 10% protein solutions using the back extrusion method of Harper et al. (1978) and Hickson et al. (1982). Back extrusion was performed on an Instron Universal Testing Machine (Model 4202, Canton, MA) equipped with a 50 N load cell using a crosshead speed of 100 mm/min. "Gel strength" or work required to penetrate the gel was calculated as the area under the force deformation curve.

The soluble protein content of both the high protein flours and the concentrates was determined using the method of Morr et al. (1985).

All functional tests were performed in triplicate.

7.Statistical Analysis

The means of the nutritional and functional property analyses were compared using a one-way analysis of variance (ANOVA). The means of all of the dry bean and soy products in a table were tested as one block. Mean separation was determined using Duncan's Multiple Range Test.

C. Results and Discussion

1. Composition

a.Protein

The composition of different protein products is shown in Table 4. (The analysis of variance for the composition of each high protein product is shown in Appendix A). The protein contents of the concentrates were around 65% while the protein contents of the bean flours were about 52% which is lower than that of the soy flour with a protein content of 56%. Sosulski and McCurdy (1987) found that the protein content of the concentrate and air classified fines from field peas was 80.3% and 47.2% respectively. The reason for the relatively low protein content of the dry bean concentrates is unknown.

Table 4. Proximate composition of navy, kidney, and soy high-protein flours and concentrates[1]

Product	Moisture (%)	Protein (%)	Ash (%)	Lipid (%)	Starch (%)
Flour					
Navy	8.1^{A}	51.9^{A}	8.2^{A}	3.3^{A}	3.6^{A}
Kidney	6.5^{B}	52.4^{A}	7.2^{B}	2.3^{A}	3.5^{A}
Soy	4.7^{C}	55.5^{A}	6.8^{B}	0.6^{B}	0.9^{C}
Concentrate					
Navy	3.6^{D}	65.6^{B}	7.8^{A}	3.6^{A}	2.8^{B}
Kidney	2.6^{E}	65.5^{B}	10.0^{C}	2.5^{A}	2.8^{B}
Soy	3.7^{D}	66.5^{B}	4.1^{D}	0.5^{B}	1.8^{C}
Standard Error of Mean	±0.1	±1.2	±0.07	±0.3	±0.04

[1]Dry Weight Basis

Mean values with different letter designations are significantly different. ($p < 0.05$)

b.Ash

The ash content of all of the flours is fairly high, ranging from 6.8-8.2%. It is known that air classification of dry bean meals tends to concentrate the minerals in the high protein fraction (Tecklenberg, et al. 1984). The mineral contents of the isoelectrically precipitated concentrates ranged from 4.1 to 10.0. These minerals may be associated with the proteins and thus were not separated during processing. Sosulski and McCurdy (1987) found that the ash content of dehulled faba bean

and field pea flours was 2.7 and 2.6, respectively, while the ash contents of their concentrates were 4.4 and 3.8%, respectively.

c.Lipid

The lipid content of the soy products was lower than that of the corresponding bean products probably due to extraction of oil from the flour prior to concentrate production. Sosulski and McCurdy (1987) found that the lipid content of high protein flour from field peas and faba bean was 3.7 and 2.8% respectively while the corresponding concentrates contained 1.7% (field peas) and 2.0 (faba beans).

d.Starch

Most of the starch was removed by the air classification and the isoelectric precipitation leaving approximately 3.6% in the navy and kidney high protein flours and 3% in the dry bean concentrates. This is similar to the results achieved by Sosulski and McCurdy (1987) in which air classified faba bean proteins contained 3.7% starch and a faba bean concentrate contained 1.8% starch.

2. Nutritional Characteristics of Bean Products

Digestible protein and methionine content of the proteins of the navy and kidney bean products are shown in Table 5. (The analysis of variance for each of these nutritional properties is shown in Appendix A).

Table 5 Digestible protein and methionine content of bean products.

Product	Digestible Protein[1] (%)	Methionine (mg/100mg Protein)
Navy Meal	78.7[A]	1.4[A]
Kidney Meal	79.9[A]	1.2[B]
Navy High-Protein Flour	81.1[AB]	1.3[A]
Kidney High-Protein Flour	78.2[A]	1.1[B]
Navy Concentrate	79.6[A]	0.9[B]
Kidney Concentrate	83.9[B]	1.1[B]
Standard Error of Mean	±0.87	±0.002

[1] Dry Weight Basis

Mean values with different letter designations are significantly different. ($p < 0.05$)

a.Digestible Protein

The proteins were 78-84% as digestible as the casein control. The digestibilities of the different dry bean

products were not significantly different except for the kidney concentrate which had a higher digestibility.

b.Methionine Content

The navy meals had higher methionine contents than the kidney meals and the content of both of these materials was not affected by the air-classification procedure. The methionine content of the kidney concentrate was not different from that of the meal and the high protein flour. There was a decrease in methionine content during the preparation of the kidney concentrates. It is possible that exposure of the navy bean proteins to alkali during the isoelectric precipitation caused a loss of methionine which resulted in the same methionine content as the kidney concentrate.

The methionine contents of the bean products were similar to those found by other researchers. Bahnassey et al. (1986) found that the methionine content of navy and pinto bean flours was 1.14 and 1.39 g/100g protein, respectively, while concentrates prepared from these flours using the method of Fan and Sosulski (1974) had methionine contents of 1.35 and 1.37 g/100g of protein, respectively. Sosulski and McCurdy (1987) studied the amino acid contents of isolates prepared from field peas, faba beans, and soybeans by isoelectric precipitation. They found that the methionine content of these products

were: 1.5g/100g protein for the soybean isolate, 0.9g/100g protein for the field pea, and 0.8g/100g protein for the fababean isolate. This indicates that the methionine content for products prepared by isoelectric precipitation can be quite low. They also reported that methionine was the limiting amino acid in both the fababean and field pea isolate. Onuma-Okezie and Bello (1988) found that the methionine content of isolates (0.87g/100g) and concentrates (0.94g/100g) from winged beans was essentially the same as winged bean flour (0.92g/100g), indicating that the alkali extraction of the proteins at pH 10 and pH 12 had little effect on the amino acid composition. It would have been desirable to have determined the cysteine contents of the protein products since one-half of the requirements for methionine is usually assumed to be supplied by cysteine.

3. SDS-Gel Electrophoresis

SDS-PAGE of the navy and kidney meals, high-protein flours and the concentrates revealed that there was very little difference in the protein distribution between the three different products (Figure 2). Examination of the photograph showed that the protein products all had major triplet bands consisting of subunits with molecular weights of 47, 45, and 43 kd. All of the bean products also had major doublet bands which consisted of subunits

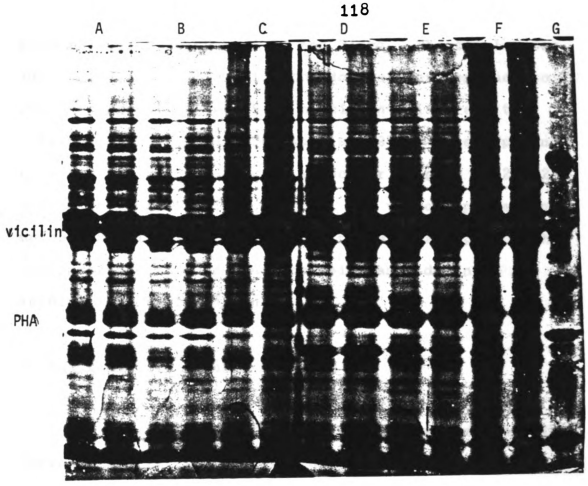

Figure 2. SDS-PAGE of Navy and Kidney Bean Meals, High Protein Flours, and Isoelectrically Precipitated Concentrates.

(A): Kidney Meal; (B): High Protein Kidney Flour;

(C): Kidney Concentrate; (D): Navy Meal;

(E): High Protein Navy Flour; (F): Navy Concentrate;

(G): Standards

with molecular weights of 32 and 31 kd. The triplet was identified as vicilin while the doublet corresponded to phytohemagglutinin, which is consistent with results obtained by Bollini and Chrispeels (1978). These products also had minor triplets with molecular weights of 28, 27, and 26 kd. Results indicate that the various processing methods had little effect on the proteins except for the disappearance of some minor protein bands during isoelectric precipitation.

4. Functionality of Bean Products

The results of the functionality tests are shown in Tables 6 and 7. (The analysis of variance for the functional property results shown in Tables 6 and 7 is given in Appendix A).

a.Soluble Protein

The dry bean high protein flours were not very soluble in aqueous solutions. This is probably because much of the protein in the flours is still bound up with the starch and other materials in small particles of insoluble bean cotyledons. The lower soluble protein content for the soy high protein flour may reflect slight structural differences between the proteins, or more probably, a more severe processing history for these

Table 6. Gelation and emulsification properties and soluble protein content of high protein products at pH 6.5

Product	Gel Strength (N-mm)	Soluble Protein[1] (%)	Emulsification (ml oil/mg prot)
Flour			
Navy	9.1[A]	25.4[A]	0.12[A]
Kidney	9.6[A]	29.0[A]	0.10[AB]
soy	2.9[B]	19.8[B]	0.08[B]
Concentrate			
Navy	9.5[A]	48.6[C]	0.12[A]
Kidney	10.0[A]	35.9[D]	0.11[A]
Soy	12.9[A]	7.8[E]	0.11[A]
Standard Error of Mean	±0.87	±1.61	±0.28

[1] Dry Weight Basis

Mean values with different letter designations are significantly different ($p < 0.05$)

proteins (i.e. defatting and spray drying). The differences in processing are reflected in the soluble protein results for the concentrates. The navy and kidney concentrates were freeze-dried while the soy concentrate was probably spray-dried. Spray-drying involves high temperature drying of the protein solution while in freeze drying, the free water is removed by sublimation. The difference in soluble protein between the navy and kidney concentrates was statistically significant, but the reason

for it is unclear because the two bean varieties are very closely related and should thus be equally affected by the isoelectric precipitation procedure.

b.Gelation

The high protein navy and kidney flours had a significantly higher gelation ability than the soy flour. The difference in gel strength between the high protein bean flours and the soy flour is probably related to the differences in soluble protein content which are also seen Table 6. The higher gel strengths of the dry bean high protein flours is probably not related to the influence of starch. It is known that gluten and wheat starch form complexes at about 60°C during starch gelatinization which either interfere with the transfer of water into the starch serving to delay gelatinization or prevent an increase in starch solution consistency. (Olkku and Rha, 1978; Eliasson, 1983). Presumably, any starch-protein complexing in the high protein flours would have prevented gel formation entirely because of interference with the denaturation of the proteins.

All of the concentrates appear to have the same gelation ability however the soy concentrate had a much coarser texture than the other gels and may have aggregated rather than formed a true gel. In these products, the higher protein contents may have compensated

for a lack starch to form firm gels. It should also be noted that it was difficult to get consistent gels from the soy concentrate.

c.Emulsification

Navy and kidney bean flours emulsify significantly more oil on a constant protein basis than soy flour (Table 6) while the protein concentrates had the same emulsion forming ability. Soy proteins are poor at forming emulsions because of their rigid structure which inhibits the unfolding of the protein at oil/water interfaces (Franzen and Kinsella, 1976; Kim and Kinsella, 1987a) and is the reason the soy flour had a lower emulsification capacity than the other flours. The soy concentrate has the same emulsification ability but a lower soluble protein content because of partial denaturation of the protein during isoelectric precipitation. This change may not have been severe enough to cause a decrease in soluble protein content but did affect the nonpolar regions of the protein molecules where the oil would bind. The navy and kidney high protein flours have essentially the same composition and one would expect similar emulsification capacities since they have the same soluble protein content.

Table 7. Foaming properties of high-protein bean products[1]

Product	Average Foam Stability (% Initial Volume)	Specific Volume (ml/g)	Volume Increase (%)
Flour			
Navy	93^{AB}	1.6^{A}	40.1^{A}
Kidney	92^{AB}	1.7^{A}	55.3^{A}
Soy	94^{AB}	1.3^{B}	20.3^{B}
Concentrate			
Navy	84^{B}	1.5^{A}	39.5^{A}
Kidney	85^{B}	1.6^{A}	49.9^{A}
Soy	97^{A}	1.4^{B}	16.4^{B}
Standard Error of Mean	±7.5	±0.01	±10.3

[1]ANOVAs for these properties are in Appendix A.

Mean values with different letter designations are significantly different ($p < 0.05$).

d.Foaming

Navy and kidney bean flours and their concentrates form significantly higher volume foams than comparable soy products under the conditions used in these tests (Table 7). The higher foam volume and the lower foam stability of the dry bean products as compared to the soy products may be an indication of compositional or structural differences between the proteins which give the dry bean proteins a less rigid structure than the soy proteins.

The dry bean concentrates formed foams which are less stable than that of the soy concentrate (Figure 3). The differences in foam stability are probably related to the low protein solubility of the soy concentrate as compared with the dry bean concentrates. These proteins may be so denatured by processing that they don't refold very readily, leading to a very stable foam once it has been formed.

The high protein flours form foams which have the same stability as the soy flour. This is probably a reflection of the fact that the soy flour has the a much higher soluble protein content than the soy concentrate and thus will more readily refold at the air/water interface.

An attempt was made to measure foam ability and stability using the method of Phillips et al. (1987). This was unsuccessful because neither the soy flour nor the navy flour would produce foams of any substantial volume. To produce the foams, it was necessary to use the higher torque produced by a Waring blender in the method of Sathe and Salunkhe (1981a). Because such a high torque is required, these protein products would probably not be used solely for their foam forming ability. However, they could prove acceptable if their structure was modified by methods such as partial proteolysis or succinylation which have been proposed for soy beans (Chen and Morr, 1985; Kim and Kinsella, 1987a; Kim and Kinsella, 1987b).

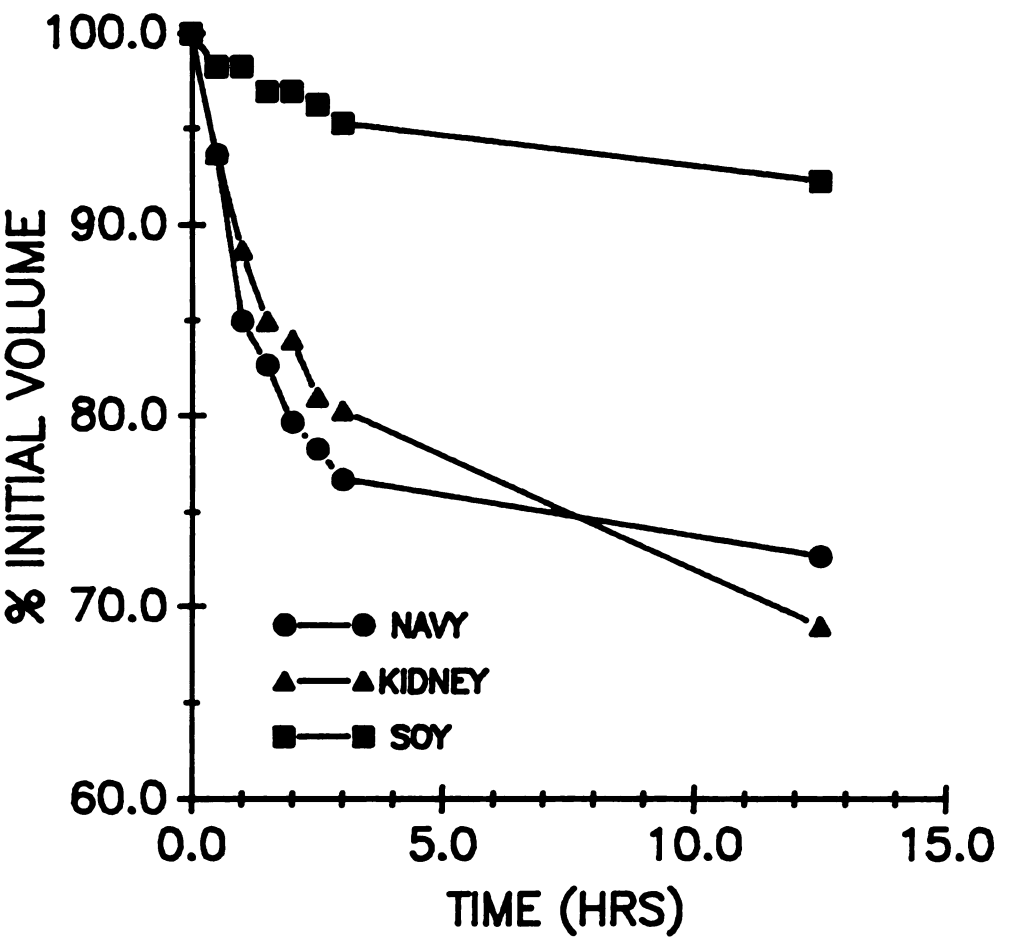

FIGURE 3. FOAM STABILITY OF DRY BEAN AND SOY CONCENTRATE AS DETERMINED BY THE METHOD OF SATHE AND SALUNKHE (1981a)

D. Conclusions

The results of this study indicate that the functional properties of the navy and kidney bean high protein flours and concentrates are generally comparable to those from soy beans. The high protein flours from dry beans produced gels with gel strengths significantly higher than the soy and the dry bean products had significantly higher soluble protein contents than the soy products. This was probably due to the defatting of the soy high protein flour and concentrate and the spray drying of the concentrate. The high protein dry bean products also formed foams with significantly higher volumes than the soy bean products when whipped under high torque conditions. Conversely, the foams formed by the dry bean concentrates are less stable than those of the soy. This may be important when aqueous solutions of these products are accidentally subjected to high sheer and form undesirable foams. Processing of the dry bean meals into high protein flours and concentrates did not significantly change the protein digestibility while only the methionine content was significantly affected by concentrate production. While the protein products from dry beans may not entirely replace soy proteins, they might be better for use in certain applications.

VII. STUDY III. EFFECT OF pH and NaCl CONCENTRATION ON PROGEL FORMATION AS DETERMINED BY DYNAMIC RHEOLOGICAL PROPERTIES OF A NAVY BEAN PROTEIN CONCENTRATE

A. Introduction

The ability of proteins to form gels is important in many food systems. For soy proteins, the ability to form gels is important in tofu in which it is the major ingredient and in processed meats in which it replaces more expensive muscle proteins and must contribute to product texture. (Voutsinas et al., 1983; Kinsella, 1976). The proteins from dry beans and similar plant species have been shown to form gels similar to those of soy proteins (Fleming et al. 1975; Sathe and Salunkhe, 1981a; Sathe et al. 1982).

A mechanism for the gelation of soy protein globulins was proposed by Catsimpoolas and Meyer (1970). In it, the gelation process was thought to involve the irreversible conversion of a protein sol to a viscous progel by thermal destruction of the quaternary structure. In the second step, the progel was cooled, allowing the formation of intermolecular bonds which in turn helped to form the gel structure. For thermally reversible gels, the bonds formed were theorized to be mainly hydrogen bonds the formation of which is favored at low temperatures. Hydrophobic bonds are strengthened by increasing temperature and these may contribute to the viscosity of

the progel state. Babajimopoulos et al. (1983) studied the effect of various anions on the gelation properties of soy protein dispersions during heating and cooling. Results of this work indicated that the major forces involved in the gelation of soy protein were hydrogen bonding and van der Waal's forces with only minor involvement of hydrophobic interactions. Utsumi and Kinsella (1985) studied the effects of different reagents of the formation and maintenance of soy gel structure. Results for gels formed from soy isolate indicated that hydrogen bonds and hydrophobic interactions were involved in the formation of the gels while disulfide and hydrogen bonds contributed to the maintenance of the gel structure.

Most gelation work has involved testing of separate gels after heating for a particular temperature and time. It is desirable to follow the course of gelation as a protein is heated since it will give a dynamic (continuous) picture of gelation. In dynamic testing two moduli are determined: 1. G'(the storage modulus) which is a measure of energy stored due to elastic deformation of the sample, and 2. G" (the loss modulus) which measures the energy dissipated as heat due to viscous flow in the sample. From these two moduli, two other factors may be calculated:

1. G* (the complex modulus) = $[(G')^2 + (G")^2]^{1/2}$
2. Tan δ (loss tangent) = G"/G'

G* is proportional to the total energy required to deform the material while Tan δ compares the liquid/solid characteristics of a protein solution. In principle, as gel networks form, the sample becomes more elastic in nature and G′ values will rise while the Tan δ values fall (Beveridge et al., 1984).

Very little work has been done using non-destructive dynamic testing to determine the gelation characteristics of vegetable proteins. Van Kleef (1986) determined the dynamic rheological properties of a soy isolate and the 11S globulin as the protein materials were heated from 50-98°C in water containing 25% isolate at pH 5.5. Upon heating, an increase in G′ was noted around 70°C followed by a second transition about 90°C. The authors attributed the first increase in G′ to the gelation of the 7S protein while the second transition was due to gelation of the 11S protein. Beveridge et al. (1984) used dynamic testing to follow the gelation of Promine D (a commercial soy protein concentrate) at pH 6.8. The G′ of the soy concentrate solution increased as both the heating temperature and solids content increased. Scanning Electron Micrographs (SEM) of the soy gels showed them to consist of many large particles adhering together and apparently embedded in a background matrix. Gill and Tung (1978) measured the steady shear flow behavior and dynamic rheological properties of gels made from the 12S glycoprotein of canola. The viscoelastic response was

measured as Tan δ as a function of oscillatory frequency . At pH 2, the gels had very high Tan δ values and low values at pHs of 9.2 and 10. Gels formed at pH 6.0 had viscoelastic properties similar to the gels formed at higher pHs. Measurement of the apparent viscosities of the different gel solutions indicated that the strongest gels were formed at high pH and ionic strength.

Very little is known about the gelation ability of dry bean proteins because it has not been investigated in depth. Also, the dynamic rheological properties of dry bean proteins have not been studied. This purpose of this investigation was to use dynamic rheological testing to determine the effect of NaCl and pH on progel formation of a navy bean protein concentrate (NPC). The dynamic rheological properties were correlated with changes in protein structure measured by hydrophobicity and solubility.

B. Materials and Methods

1. Navy Concentrate Production

Michigan navy beans were dehulled and ground into a flour. The proteins from the flour were then extracted using the method shown in Figure 4 to produce the isoelectric concentrate. The concentrate was stored in

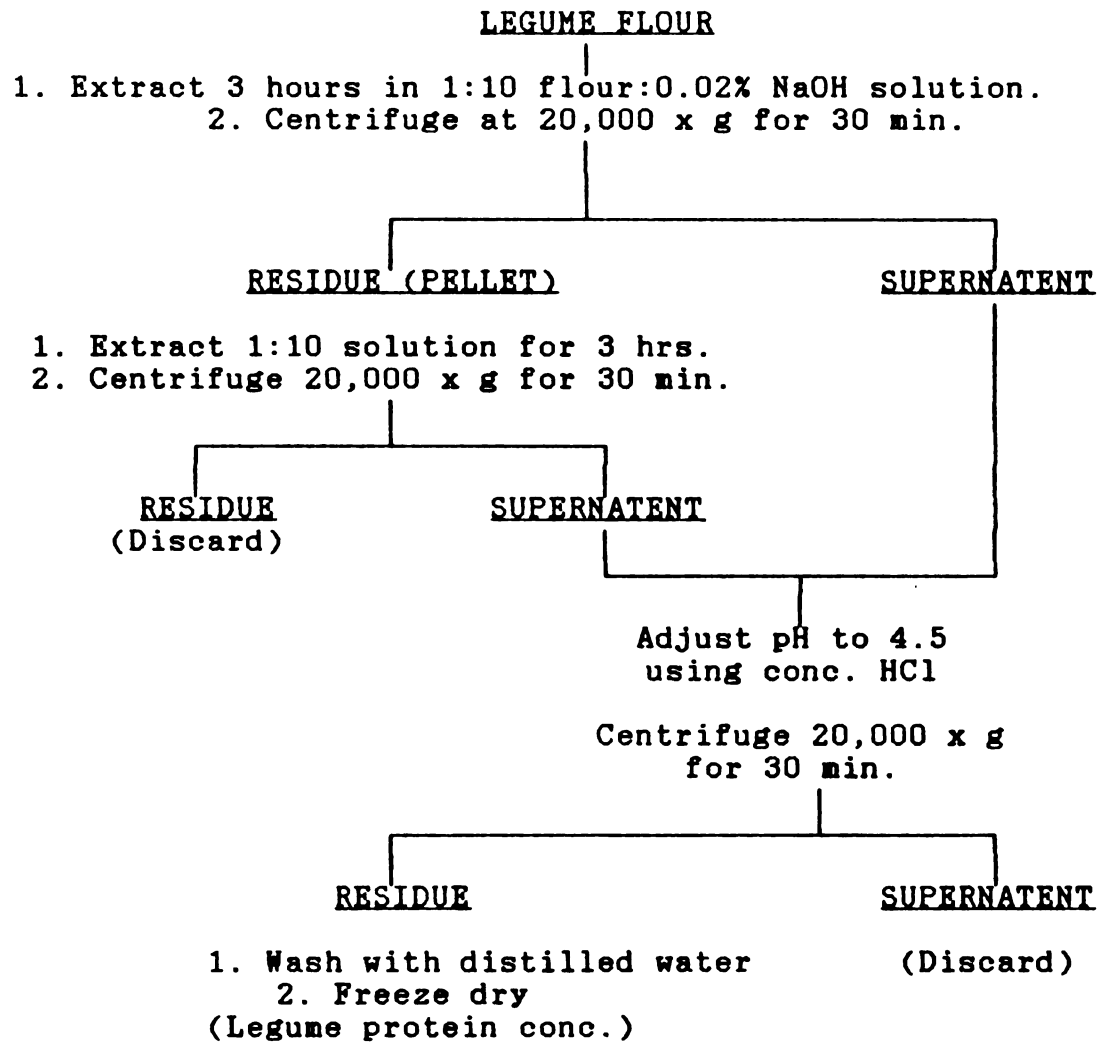

Figure 4. Method of production for isoelectric precipitate from navy bean flour

brown jars which were placed in a desiccator and kept in a freezer at -20°C.

2. Compositional Analysis

The protein content of the navy bean flour and concentrate was determined using AOAC Method 24.038-24.040 (AOAC, 1984). The protein content was determined on a dry weight basis using a nitrogen conversion factor of 6.25. Moisture was determined using AACC Method 44-40 (AACC, 1983) and ash was determined using AACC Method 08-01 (AACC, 1983) which was modified slightly to obtain complete ashing of the high protein materials. The crucibles containing dried samples from the moisture determination were wet ashed by saturating the samples with HCl, digested until a gray ash formed, and put in the muffle oven overnight. The composition of the NPC was determined on a dry weight basis. The soluble protein content of the concentrate and navy bean flour in 0.2M NaCl, pH 7.0 was determined using the method of Morr et al. (1985).

The starch content of the concentrate was determined by hydrolyzing the starch to glucose using the method of Englyst et al. (1983). Glucose content of the digested samples was determined using the method of Budke (1984).

Soluble protein was performed a total of six times: 2 separate samples were made up and the soluble protein content of each sample was determined three times.

Protein, starch, moisture, and ash were determined in triplicate.

3. Electrophoresis of Bean Products

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the navy bean concentrate and the proteins soluble at pH 7.0 was performed on 12% (0.25% bis) acrylamide running gels with a 4% stacking gels using the system of Laemmli (1970). Electrophoresis was performed with a Hoeffer Vertical Electrophoresis unit (Model SE 600; Hoeffer Scientific Instruments, San Francisco, CA) using a constant voltage power supply (Heathkit Model 1P-17, Benton Harbor, MI). Ten µl of a 0.25% (w/v) protein solution were put on the stacking gel. A constant current of 30 mA was applied until the proteins migrated into the running gel and then the current was increased method to 60 mA until the bromophenol blue tracking dye reached the bottom of the running gel. The gels were removed and stained for 6 hr in 0.125% Coomassie Blue in 50/10/40 (v/v/v) methanol/acetic acid/water. The gels were destained in 7/5/88 (v/v/v) acetic acid/methanol/water until clear.

The relative mobility (RM) of each protein subunit was calculated and the molecular weight (MW) was estimated from a standard curve of RM vs. MW prepared using a mixture of low molecular weight (14.2-66 kilodalton)

proteins (Sigma Chemical Corp., St. Louis, MO). The amount of protein present in each band on the gels was determined using a Shimadzu Dual Wavelength Thin-Layer Chromato Scanner (Model cs-930, Kyoto, Japan). The protein bands were identified by their subunit molecular weights.

4. Preparation of Hydrophobicity, Soluble Protein, and Dynamic Rheological Samples

Solutions of 10 mg of protein/ml for the solubility tests, 1 mg/ml for the hydrophobicity tests, and 120 mg/ml for the dynamic rheological tests were prepared at pH 4.5, 6.0, 7.0, and 8.0 with 0.2M NaCl and also at 0.0, 0.2, 0.4, and 0.6M NaCl with a pH of 7.0.

For the solubility and hydrophobicity testing, 10 ml aliquots were placed in 15 x 105 mm screw cap test tubes and the test tubes were placed in a test tube rack. This test tube rack was then placed in a water bath equipped with a programmable temperature controller. The programmer was set to go from an initial temperature of 30° to 95°C at a rate of 1°C/min. Samples were removed at 30, 50, 70, 80, 90, and 94°C.

5. Dynamic Rheological Testing of Navy Concentrate

A Rheometrics Fluid Spectrometer Model 8400 (Rheometrics Inc., Piscataway,NJ) was used to determine the dynamic rheological properties of the NPC. A 120mg/ml protein was found in a preliminary study to be the minimum protein concentration needed to form a gel. Solutions of this protein concentration were made up at the desired pH and NaCl concentration and held at 4°C overnight. Before dynamic testing, the pH of the sample was readjusted if necessary. For the test itself, 3 ml of sample were placed into the sample cup of a Rheometrics Fluid Spectrometer. The machine was set to perform dynamic testing using a cone and plate arrangement. The cone was lowered into the sample to a preset height of 35 µm. above the plate. A constant temperature water bath with a temperature programmer was used to heat the sample at a constant rate of 1°C/min from 50 to 90°C. Ethylene glycol was used as the heating medium because of its relatively low volatility at high temperatures. All of the dynamic tests were started at 50°C because the results of the hydrophobicity testing and the solubility testing indicated that no transitions occurred before that temperature. The sample was placed in the 50°C bath and allowed to equilibrate for 5 min. Following equilibration, the sample was heated and the G′ and G″ of

the sample recorded against time until there was no change in either loss or storage modulus. The tests were conducted until two reasonably consistent results were obtained from the same sample. From these data, G* and tan δ were calculated.

a.Strain and Frequency Sweeps

The sample strain and the frequency of oscillation conditions under which the fluid spectrometer tests were to be run were determined before the actual testing began. Frequency sweeps were conducted at 50, 75 and 90°C at frequencies from 1 to 100 rad/sec at strains from 0.1-10%. Strain sweeps were conducted from 0.01 to 100% at a frequencies ranging from 1-15. An outline of the tests conducted is given in Table 8.

6. Hydrophobicity of Navy Protein Concentrate.

The hydrophobicity of the heated NPC solutions was determined by reacting them with ANS (8-anilino-1-naphthalene sulfonic acid). After heating, the protein solutions were diluted with 0.2M phosphate buffer to obtain protein concentrations of 0.6, 0.10, 0.05, and 0.01 mg/ml. A reference solution consisting of 0.1 mg/ml BSA

in 0.1 M buffer at pH 7.0 and an ANS stock solution (8mM ANS in 0.1M phosphate buffer, pH 7.0) were also prepared.

Table 8. Outline of strain and frequency sweeps conducted to determine conditions for testing on the fluid spectrometer.

Frequency Sweeps (1-100 rad/sec)					
Temp.	Strain	Temp.	Strain	Temp.	Strain
50	0.1	75	0.1	90	1.0
	1.0		1.0		5.0
	5.0		5.0		10.0
			10.0		

Strain Sweeps (0.01-100 %)					
Temp.	Freq	Temp.	Freq	Temp.	Freq
50	1.0	75	5.0	90	5.0
	5.0		10.0		10.0
	10.0		15.0		
	15.0				

The fluorescence intensity (FI) for each solution was measured on a Varian Spectrofluorometer (Model SF-330; Varian Assoc., Palo Alto, CA) at an excitation wavelength of 366 and an emission wavelength of 481. The FI was standardized by mixing 2 ml of methanol and 10 µl of the ANS stock solution in a cuvette, allowing it to react for

1 min, and adjusting the reading on the fluorometer to give an FI of "150".

The FI of each protein concentration was determined by adding 10 µl of the ANS stock to 2 ml of each dilution, mixing, allowing the reaction to occur for 1 min and then measuring FI. The net FI of each dilution was calculated by subtracting the FI of each solution without the ANS from that of the solution with the ANS. The net FI of a 2 ml aliquot of the BSA solution was determined periodically to assure that the fluorometer was functioning properly. The slope of FI vs protein concentration (mg/ml) as determined by linear regression was used as a measure of absolute protein hydrophobicity. From this data, a plot of absolute hydrophobicity vs heating temperature was constructed (these are shown in Appendix B).

A relative hydrophobicity value for the proteins at each temperature was calculated to make comparison between samples easier. The relative hydrophobicity was calculated by converting all of the absolute hydrophobicities within a sample treatment to decimal fractions of the absolute hydrophobicity at 94°C.

7. Solubility of Navy Concentrate Proteins

The solubility of the heated protein solutions was determined using the method of Morr et al. (1985). The soluble protein content of each sample was determined by

calculating the amount of protein in the supernatent and dividing it by the total amount of protein present in each sample (plots of soluble protein content vs. heating temperature are shown in Appendix B). The amount of insoluble protein in each sample was calculated by subtracting the amount of soluble protein from 100.

C. Results and Discussion

1. Composition of Navy Concentrate

The composition of the concentrate is shown in Table 9. There is relatively little starch in the concentrate compared to the normal starch content of dry beans, indicating that starch had relatively little effect on the progel formation of the concentrate. The extraction procedure removed most of the ash from the flour and also increased the soluble protein content slightly. This increase in protein solubility may be due to: 1. freeze-drying increasing the water dispersibility of the proteins or 2. selection of the most soluble proteins in the bean flour by the extraction procedure. There may be interfering substances in the flour which inhibit protein solubility such as protein/starch complexes which have been shown to form in bread flour (Olkku and Rha, 1978; Eliasson, 1983). The solubility

Table 9. Composition of navy flour and concentrate[1]

	Concentrate (%)	Flour (%)
Moisture:	2.7	6.1
Protein:	85.4	27.2
Soluble Protein	71.1	64.4
Ash:	2.6	4.1
Starch:	2.4	44.6[2]

n=3

[1]Dry Weight Basis

[2]Derived from Tomkinson (1986)

test was run only at pH 7.0 since this is close to the pH at which most of the progel formation testing was done.

2. Electrophoresis

The diagram of the SDS-PAGE slab gel (Figure 5) indicates vicilin and phytohemagglutinin (PHA) and most of the minor proteins were present in the same relative proportions in both the navy bean flour and concentrate. Some of the highest and lowest molecular weight protein fractions present in the flour were lost during concentrate production. These empirical results are confirmed by densitometer scans of the gel slabs (Table 10).

Table 10.Protein composition of navy concentrate, flour, soluble concentrate proteins, and soluble flour proteins

Material	Protein Fraction (% of Total)		
	60 kd	vicilin	PHA
concentrate	10.2	54.8	4.6
flour	10.5	44.3	9.2
soluble concentrate	2.7	75.1	3.3
soluble flour	25.3	42.0	0.0

The major proteins in the concentrate were more soluble than those in the flour as expected from the results of the soluble protein determination. In the concentrate lane, the three major subunits of the vicilin can be seen clearly while in the soluble protein lane (containing the same protein concentration), there is so much of each subunit present that the subunits tend to blend together instead of forming three distinct bands.

The vicilin fraction, which is the major protein fraction in navy beans, comprises only 44% of the total protein in the flour while in the concentrate it is about 54% of the protein. There is also a protein fraction in both the flour and the concentrate which has a molecular weight of about 60 kd. The presence of this protein fraction was noted by Bollini and Chrispeels (1978) and

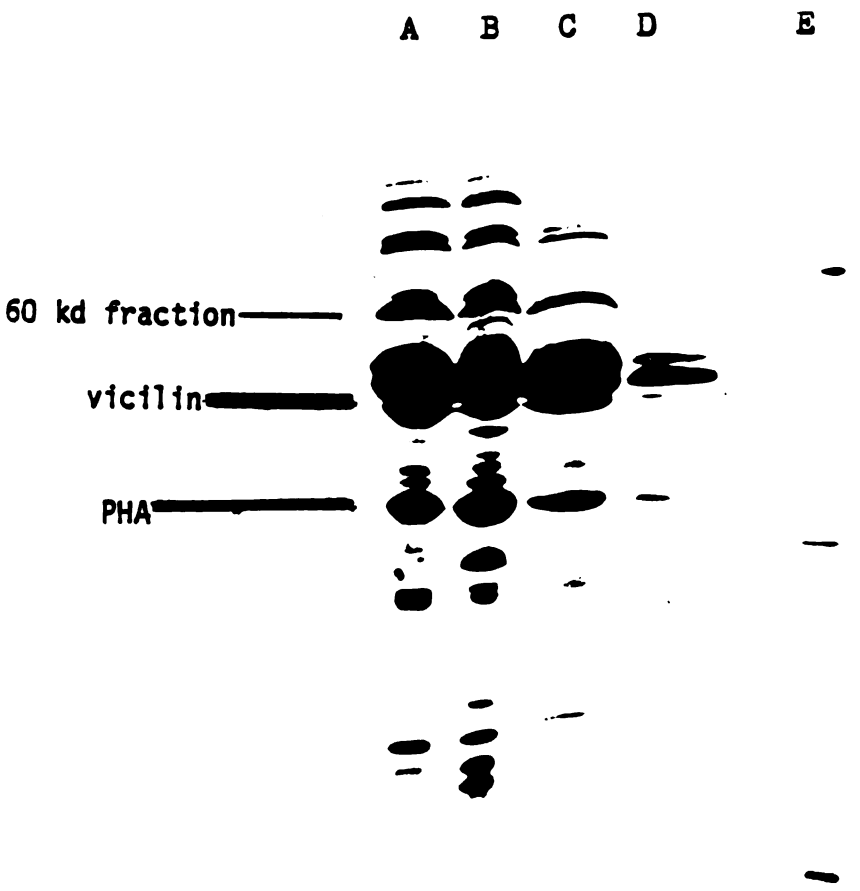

Figure 5. SDS-PAGE of Navy Bean Flour, Navy Protein Concentrate, Soluble Concentrate, and Soluble Flour Proteins.
(A): Navy Protein Concentrate; (B): Navy Bean Flour
(C): Soluble Concentrate; (D): Soluble Flour
(E): Standards

Barker et al.(1976). Derbyshire and Boulter (1976) isolated a fraction from dry beans with a molecular weight of 62 kd which they identified as a breakdown product of an 11S protein. In the flour, the 60 kd fraction is about 10% of the protein, slightly more than the amount of PHA present. In the concentrate, the 60 kd fraction is still about 10% of the protein present while the amount of PHA decreased to about 4.6%. This loss in the PHA was accompanied by an increase in the amount of vicilin present in the concentrate. Isoelectric precipitation may provide a method for reducing the anti-nutritional factors in beans by reducing the amount of PHA present.

In the densitometer scan of the soluble flour protein, no PHA was detected in the soluble flour protein while there was a small amount present in the soluble concentrate. The PHA may be associated with the carbohydrates in the flour or in some other way is more insoluble in the flour than in the concentrate.

3. Heat Stability of NPC

A preliminary study was conducted to determine the heat stability of the NPC. The change in ANS hydrophobicity as a function of length of exposure to 95°C heat is shown in Figure 6. The navy bean proteins are extremely stable to high temperatures. The proteins unfolded within the first 5 min as indicated by the large

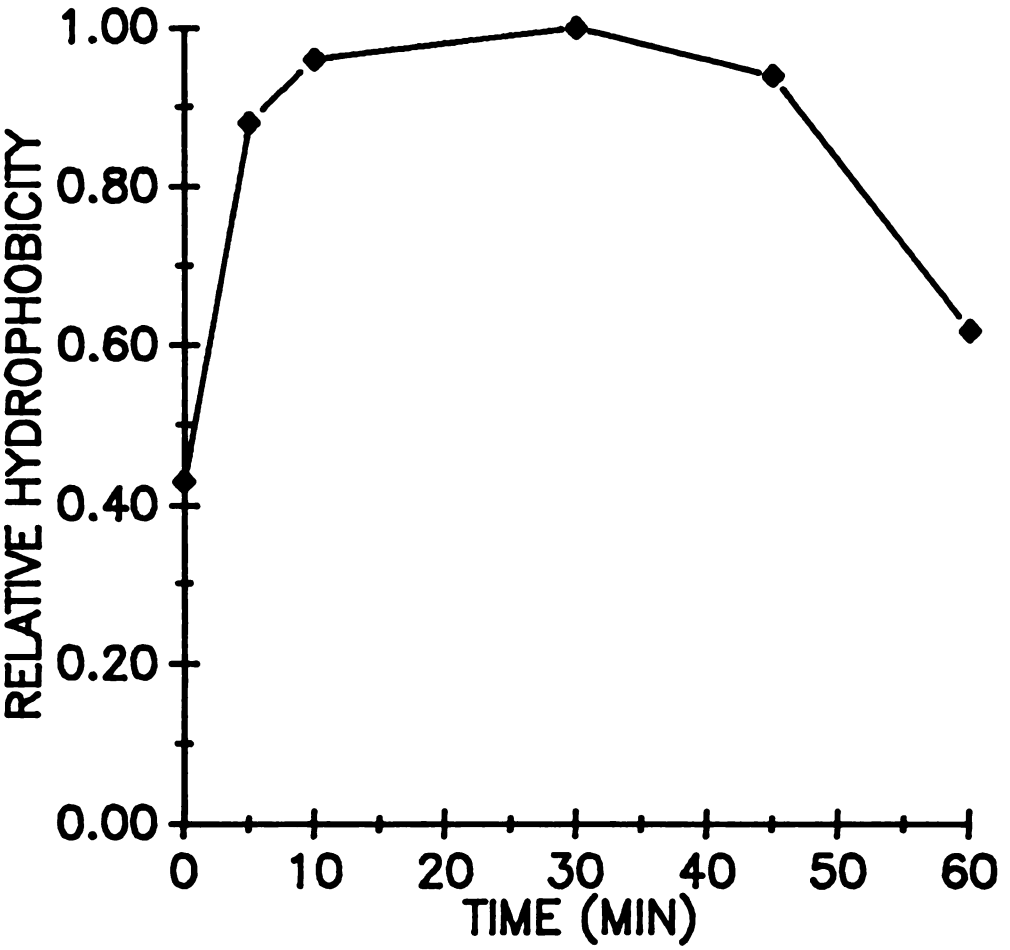

FIGURE 6. HEAT STABILITY OF NAVY PROTEIN CONCENTRATE AT 95°C AS MEASURED BY ANS HYDROPHOBICITY AT pH 7.0, 0.2M NaCl

increase in hydrophobicity. There was little change in hydrophobicity indicating that there was little unfolding or aggregation until after an extremely long exposure to the high temperatures. After 40 min, the hydrophobicity began to decrease indicating that the proteins had begun to aggregate.

4. Progel Formation of the NPC

The gelation model for soy globulins proposed by Catsimpoolas and Meyer (1970) involves the heating of a protein solution to achieve the viscous progel state followed by cooling to achieve gel setting. In this investigation only the formation of the progel was followed because equipment limitations did not allow the gelation process to be followed during cooling.

Analysis of the strain and frequency sweeps indicated that the desirable conditions for performing the dynamic tests were at a frequency of 2 rad/sec and 2% strain. In this range there was relatively little effect of either strain or frequency on the G′ and G" of the navy protein solution at 50, 75, or 90°C. Any increase or decrease in G′ or G" would be due to heating of the sample and not the test equipment. There was considerable variation in the progel formation curves between aliquots which were taken from the same sample. This variation was noted by Beveridge et al. (1984) in their study of the dynamic

rheological properties of soy protein concentrate. In this investigation, G´ values and clumping tended to increase with sample age so fresh samples were made up each day.

Gill and Tung (1978) found that aliquots of a 12S canola glycoprotein solution aged for 4 days had a higher viscosity than aliquots from an unaged sample. They also found that the stored solution tended to have a much lumpier texture than the fresh solution. They suggested that this was the result of self-association of the protein in the cold to form higher molecular weight complexes.

In general, the G* of the samples increased, levelled off, and in some cases decreased slightly. Catsimpoolas and Meyer (1970) noted this same phenomenon in the gelation of soy protein globulins. They attributed this to the formation of a "metasol" caused by heating the sample beyond the point necessary to form the progel. Van Kleef (1988) showed data in which a 25% soy isolate solution at pH 5.5 had a storage shear modulus curve in an "S"-shape.

The decrease in gel strength at high temperatures seen in some of the solutions could be the result of destruction of gel structure by the fluid spectrometer where the oscillation of the plate caused some of the protein matrix to be physically torn which released some of the water or it may be due formation of a metasol (Catsimpoolas and Meyer, 1970) Although the authors don´t

explain what causes the metasol, it might be due to a release of water caused by coagulation of protein molecules at the higher temperatures.

a. Effects of NaCl Concentration on Progel Formation

The effect of NaCl concentration on G* at pH 7.0 is shown in Figure 7. The characteristics of the curves showing the effects of NaCl on progel formation in the NPC are shown in Table 11.

Table 11. Effect of NaCl on rheological characteristics of progel formation curves of navy concentrate

Treatment	NaCl Concentration (M)			
	0.0	0.2	0.4	0.6
Temp for G* = 5000 Pa (°C)	68.5	72.7	74.0	85.0
Temp at Max G* (°C)	71.8	84.0	83.9	89.8
Start of Progel Formation (°C)	63.3	66.0	67.0	64.7
End of Progel Formation (°C)	70.2	69.9	75.0	85.7
Slope of Gel Curve	1529	871	806	220
Y-Intercept (x 10^4)	-9.8	-3.0	-5.5	-1.5

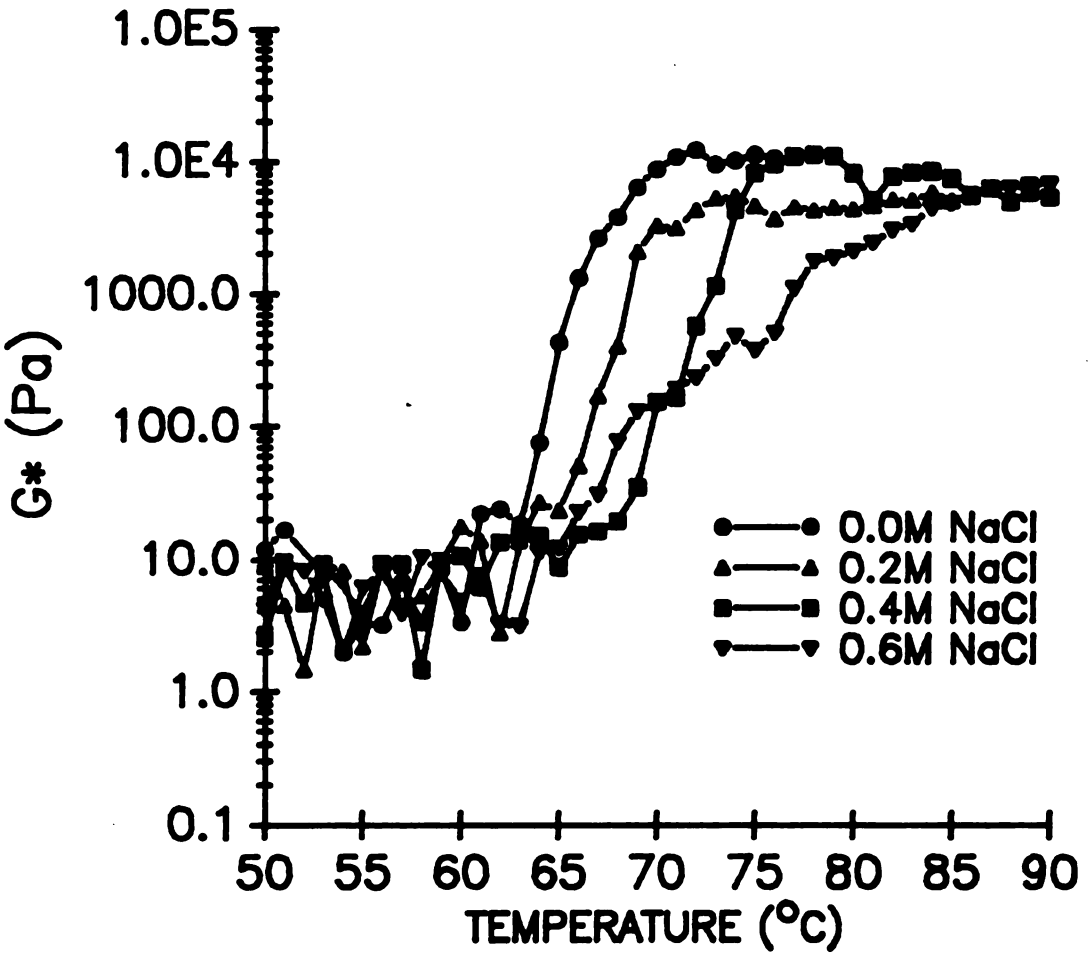

FIGURE 7. EFFECT OF NaCl ON G* OF NAVY PROTEIN CONCENTRATE, pH 7.0 HEATED FROM 50–90°C AT 1°C/MIN

When there is no NaCl added to the protein solution, there is very little, if any, stabilization of vicilin structure. At this level progel formation begins around 63° and apparently ends around 70°. This occurs because there was too great a torque on the machine at this point and the experiment was ended. The slope of the progel formation curve was about twice as great as the next nearest progel formation slope and the sample reached 5000 Pa at the lowest temperature. The high slope and low 5000 Pa temperature indicate that this solution may have experienced aggregation and coagulation of the proteins rather than formation of a true gel. This would result because an aggregated protein mixture would have more of a solid than the semi-solid texture of a gel. In this solution, the relative hydrophobicity and the amount of insoluble protein began to increase early in the heating cycle with the hydrophobicity increase starting around 30° and the insoluble protein content increase around 50°C (Figure 8). This tends to support the idea that very rapid changes occurred in protein structure which may be the aggregation and coagulation seen with the fluid spectrometer.

Studies of pea legumin (Gueguen et al., 1988) using an ultracentrifuge showed that the protein was maintained in a 12S conformation when the ionic strength was above 0.05M and the pH was above 7.0. Below that pH and ionic

strength, the quaternary structure of the protein tended dissociate and form aggregates.

The concentrate solutions at 0.2M and 0.4M NaCl begin to form progels around 64-66° but progel formation ends at 70° for the 0.2M solution and 75° for the 0.4M solution. The 0.2M NaCl solution also attains 5000 Pa at a lower temperature than the 0.4M. The delay in progel formation seen in the 0.4M solution may again be evidence of the tendency for salt to stabilize the quaternary structure of legume proteins against denaturation, possibly because of increasing electrostatic attractions between the subunits of the major proteins. Once the temperature became high enough to begin breaking ionic bonds, the 0.4M solution began to gel. In this solution (Figure 8), the increase in insoluble protein content and hydrophobicity is very gradual up to 94°C. In comparison, the hydrophobicity and insoluble protein content (Figure 8) of the 0.2M solution tended to increase rapidly from 70°-94°C. The reason for the dramatic decrease in hydrophobicity as the protein solution is increased in temperature from 30-50°C might be the result of the heat destroying some of the protein structure which is formed by electrostatic reactions in the native proteins.

The effect of stabilization of the quaternary protein structure can be seen during progel formation of the navy concentrate at 0.6M NaCl. In this solution, progel formation began about 65° which is similar to the 0.2 and

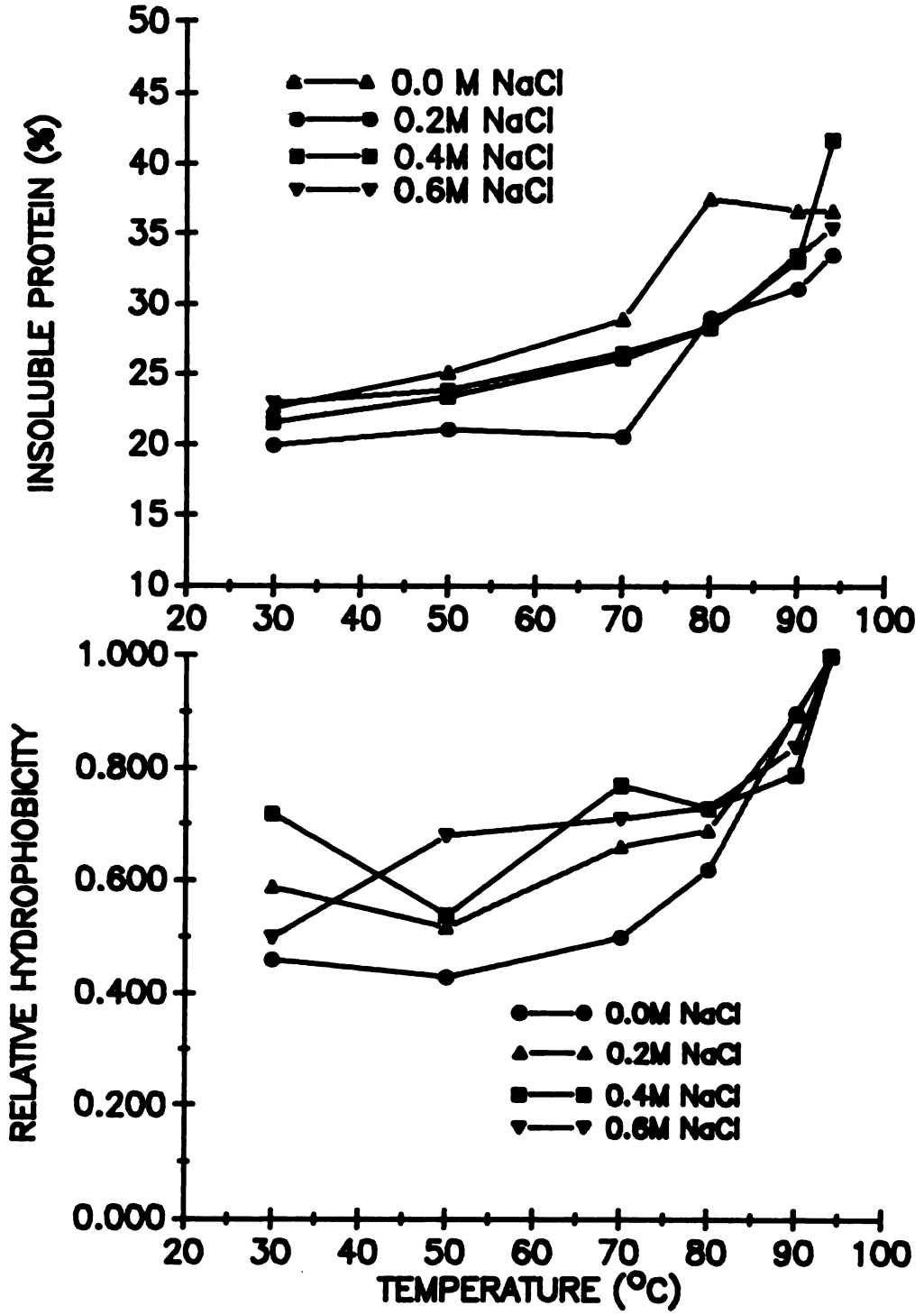

FIGURE 8. EFFECT OF NaCl ON INSOLUBLE PROTEIN AND RELATIVE HYDROPHOBICITY OF NAVY PROTEIN CONCENTRATE AT pH 7.0 HEATED 1°C/MIN

0.4M solutions but took much longer and finally ended at 86° and a G* of 5000 Pa is achieved at 85° compared to 73° for the 0.2M and 74° for the 0.4M solutions. The hydrophobicity and insoluble protein content of this solution (Figure 8) both increased very gradually during the heating process with no great increases until 94° was attained. This indicates that the structure of the protein was stabilized by the relatively high NaCl content which prevents the proteins from unfolding as readily as they normally would.

It is important to note that while the trends seen during the hydrophobicity and insoluble protein content tests support the fluid spectrometer data, the absolute values do not. The NPC solutions were subject to variability in their progel formation characteristics making it impossible to detect very small differences between the solutions. The same problem probably also occurs when making determinations of hydrophobicity and insoluble protein content making it possible to determine only trends.

The stabilization of legume proteins by salt is well documented for proteins which are related to those from dry beans. Catsimpoolas and Meyer (1970) noted that the viscosity of soy progels decreased with increasing NaCl concentrations at temperatures above 70°C indicating that progel formation was decreased in these solutions. Hermansson (1978) studied the denaturation characteristics

of soy isolate conglycinin and glycinin using a differential scanning calorimeter (DSC). She found that the denaturation temperature of conglycinin increased by 19°C and that of glycinin by 22°C as the salt concentration increased from 0.01 to 1.0M.

Using a DSC, Arntfield et al. (1986) were able to show that the denaturation temperature of both the vicilin and the legumin of faba beans increased as the ionic strength increased. At 0.0M there was no distinct transition for vicilin, while at 0.25M and 1.0M NaCl, the vicilin transition began about 70° and 80°C respectively. The vicilin transition ended about 90° in 0.25M while in 1.0M NaCl it ended about 100°C. This increase in transition temperatures is similar to the transition temperatures increase seen in this investigation for NPC at 0.2M and 0.6M NaCl. DSC scans on purified vicilin from fababeans indicated a T_{MAX} around 90° in 0.5M NaCl at an undisclosed pH with the transition beginning at 70°. Wright and Boulter (1980) performed DSC scans on 1% dry bean globulin solutions in 0.05M phosphate buffer at pH 8.0 containing 0.5M NaCl . These were found to have a T_{MAX} of 90°C similar to that of the vicilin fraction in whole dry bean meal while purified vicilin suspended in 0.003M KH_2PO_4 at pH 7.6 had a T_{MAX} of 87°C. The transition for the dry bean meal vicilin and the purified vicilin began around 82° and ended around 102°. The dry bean meal transition temperatures are higher than those

seen in this investigation for the NPC probably because of the large amount of starch present. Hohlberg and Stanley (1987) noted that a 10% (w/w) solution of purified vicilin in 0.1M phosphate buffer at pH 7.0 with 0.3M NaCl had a T_{MAX} of 109°C with a transition that began about 100°C.

The results of Wright and Boulter (1980) imply that the progel formation characteristics of the NPC are primarily those of the vicilin fraction since it is the largest component of both the total and soluble proteins. However, the proteins found in lesser amounts in the concentrate probably do affect the progel formation reaction by lowering the transition temperatures. DSC results on purified vicilin indicate that it has a higher T_{MAX} and transition temperatures than non-purified vicilin.

b.Effects of pH on Progel formation

The effects of pH on progel formation of the NPC are shown in Figure 9 and the characteristics of the curves are shown in Table 12. The transition temperatures for these solutions are lower than those reported for vicilin in dry bean meal and for purified vicilin (Wright and Boulter, 1980).

It should be noted that the heating characteristics of the NPC were determined in solutions containing 0.2M phosphate buffer. This buffer was necessary to insure

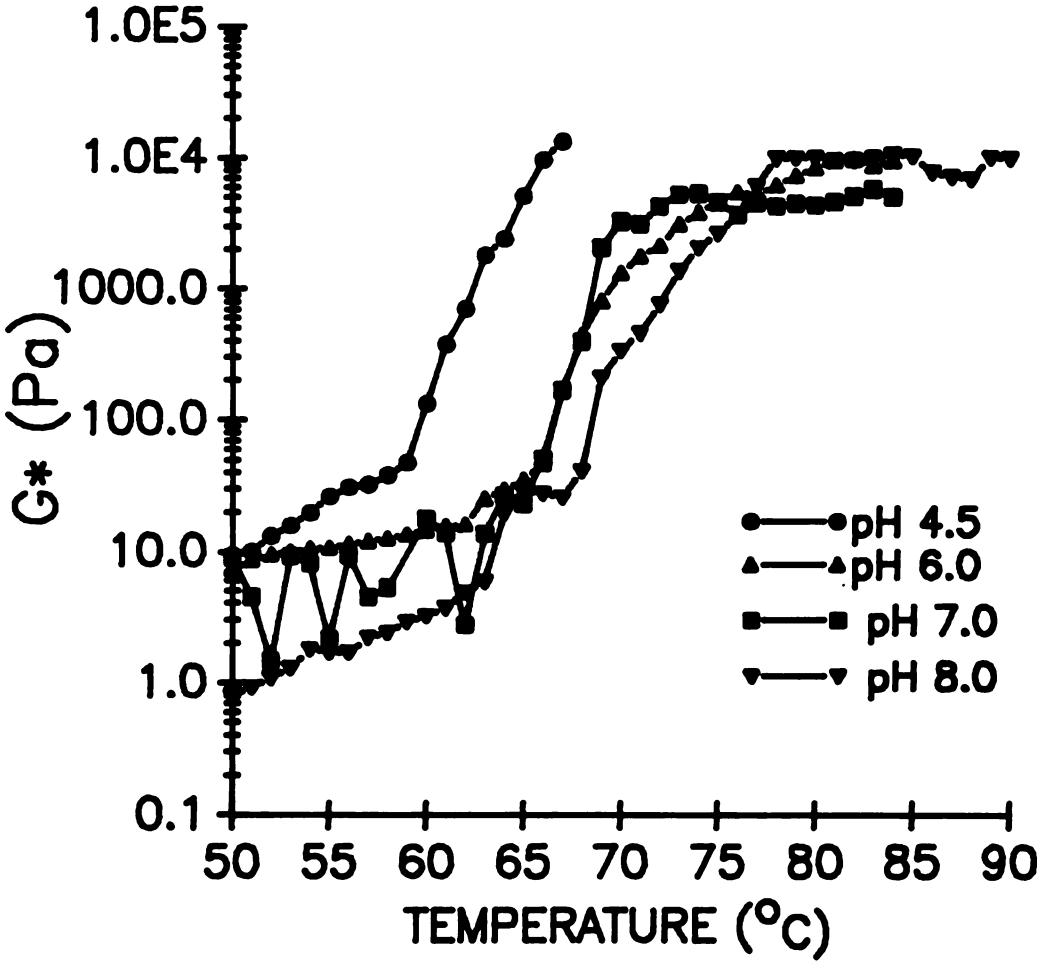

FIGURE 9. EFFECT OF pH ON G* OF NAVY BEAN CONCENTRATE, 0.2M NaCl HEATED FROM 50–90°C AT 1°C/MIN

that the pH of the solution stayed relatively constant but did add more salts to the solutions which may have contributed to stabilizing the navy proteins. Since all of the measurements were conducted in the buffer, the stabilizing effect of the phosphate probably stayed constant throughout the investigation and any differences seen in protein behavior were due to differences in the solutions themselves.

Table 12. Effect of pH on rheological characteristics of progel formation curves of navy concentrate

Treatment	pH of Solution			
	4.5	6.0	7.0	8.0
Temp for G* = 5000 Pa (°C)	65.1	72.4	72.7	76.3
Temp at Max G* (°C)	67.1	77.0	84.0	84.2
Start of Progel Formation (°C)	59.3	63.8	66.0	64.0
End of Progel Formation (°C)	67.1	73.3	69.9	79.4
Slope of Gel Curve	1532.3	664.6	871.0	612.5
Y-Intercept (x 10^4)	-9.3	-4.4	-3.1	-4.1

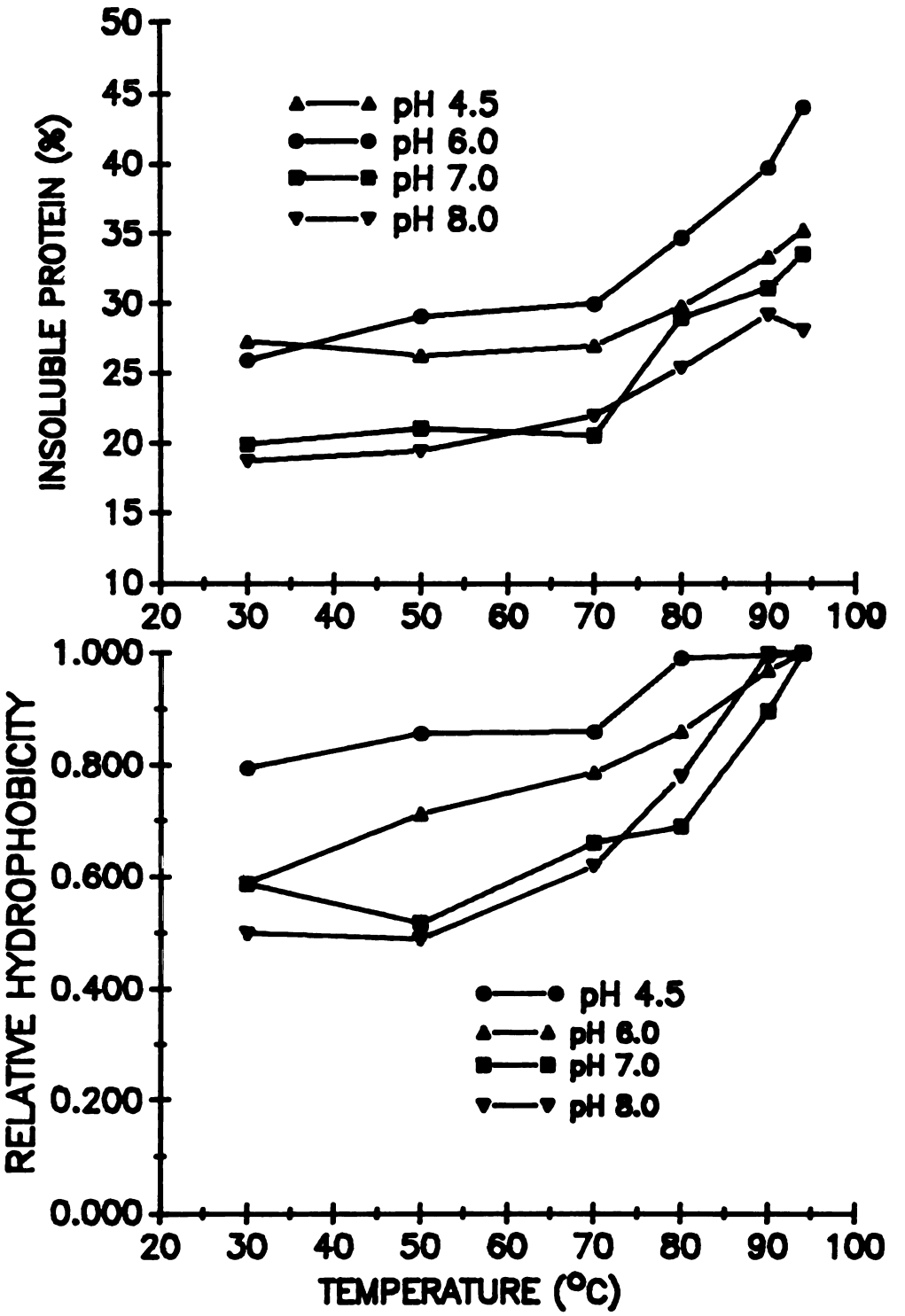

FIGURE 10. EFFECT OF pH ON INSOLUBLE PROTEIN AND RELATIVE HYDROPHOBICITY OF NAVY PROTEIN CONCENTRATE AT 0.2M NaCl, HEATED 1° C/ MIN

The effect of heating the concentrate at the isoelectric point of the vicilin is seen at pH 4.5. In this sample, the progel formation begins at 59°C and ends at 67° which is about 5° less than any of the other samples. The slope of this curve is extremely high and the sample attained a G* of 5000 Pa at 65°. The reason for the dramatic increase in G* for this sample is that the sample is probably undergoing aggregation rather than progel formation. Since the major protein is close to its isoelectric point and is known to go from a 7S to an 18S at pH 4.5 (Bollini and Chrispeels, 1978), the protein molecules are unfolding or partially unfolding and interacting inter and intramolecularly. This results in the molecules trapping very little water between them rather than unfolding and incorporating the water. This interpretation is supported by the hydrophobicity and insoluble protein results (Figure 10). At a pH of 4.5, the proteins were least soluble and the total amount of insoluble protein at this pH is greater than for the other pHs which were investigated. The proteins were stable until about 70°C when they began to denature and the amount of insoluble protein began to increase. The relative hydrophobicity also increased at about 70°C but levelled off after 80°C. This may be due to the fact that the proteins are already denatured at the start of the heating process at pH 4.5 and became resistant to heat denaturation, even at higher temperatures. It should be

remembered for these two processes that while insoluble protein content and relative hydrophobicity measure related reactions, they don't measure exactly the same reactions. The curves for these two measurements will show the same general trends but will not have exactly the same shapes.

Progel formation of the pH 7.0 sample began at 66° and ended at 70°C and yielded a G* of 5000 Pa at 73° while in the solution at pH 6.0 progel formation began at 64° and ended at 73° and achieved a G* of 5000 Pa at 72°. These figures indicate there is very little difference between pH 6.0 and 7.0 except that the G* increase at pH 7.0 occurred over a much narrower temperature range with a correspondingly higher slope. This may be due to normal sample variability rather than actual sample differences. The insoluble protein content and the hydrophobicity of the NPC at pH 7.0 (Figure 10) tends to be lower than at pH 6.0 (Figure 10) at most temperatures, indicating that the proteins are more stable at pH 7.0 than at pH 6.0. The reason for this seems to be that the hydrophobicity and insoluble protein tests at pH 6.0 did not detect the same steep rise in protein denaturation which was seen on the fluid spectrometer at the same pH. The solution at pH 6.0 had a higher initial hydrophobicity and insoluble protein content than the solution at pH 7.0 which may be the result of the protein being closer to the isoelectric point at which it is found in an 18S configuration.

Partial aggregation of the protein at pH 6.0 may lead to a protein system in which the fluid spectrometer can detect changes in protein conformation at 60-70° which are not detected by the hydrophobicity or insoluble protein tests. The transition temperatures for progel formation of the NPC pH 8.0 began at 64° and ended at 79° and the G* reached 5000 Pa at 76°. Both of these factors indicate that the proteins were more resistant to denaturation at pH 8.0 than at pH 6.0 or pH 7.0. This pH effect has not been shown by others for similar proteins. Chang and Satterlee (1981) reported that purified vicilin was more stable to heat denaturation at pH 3 than at pH 9.0. Hermansson (1978) showed that soy proteins were stable to heat denaturation at pHs from 4.0 to 9.0 with the greatest stability occurring around the isoelectric point. A pH of 8.0 may not be great enough to begin seeing denaturation of the proteins with heating which is usually associated with a pH of 9.0. Also, the NPC is not a pure compound and there might be some component interactions which tend to inhibit progel formation at pH 8.0. Stabilization of protein structure at pH 8.0 is supported by the insoluble protein test results (Figure 10) which show a very slow rate of increase and an insoluble protein content which is less than that at pH 6.0 or 7.0 for temperatures above 70°.

The solubility samples were frozen following heating and then thawed out again so that solubility measurements

could be made. The proteins in the samples were probably affected by the one freeze/thaw cycle to which they were subjected. However, as was seen in Figure 6, the proteins in the concentrate have a great deal of heat stability. Thus, one would expect that one cycle will have minimal effects on solubility as compared to the heating. Also, all of the samples at all of the conditions were handled the same way before the protein content was determined making any effect of freezing/thawing constant for all of the samples which did, after all, show distinct differences in solubility. If freezing/thawing were the predominant effect, one would expect that the solubility of all the samples would be about the same.

5. Loss Tangent (Tan δ) of NPC

In Figure 11, Tan δ values are relatively constant for all pHs from 65°C to 90°C and the same general trend is apparent in Figure 12, although it appears for the higher NaCl concentrations, that the progel formation did not really become apparent until about 70°C. The situation is completely different for the solution at pH 4.5 and for the solution containing 0.0M NaCl (Figure 13). A constant Tan δ indicates that sample is neither becoming more like a solid or more like a liquid but that its physical condition is remaining relatively constant.

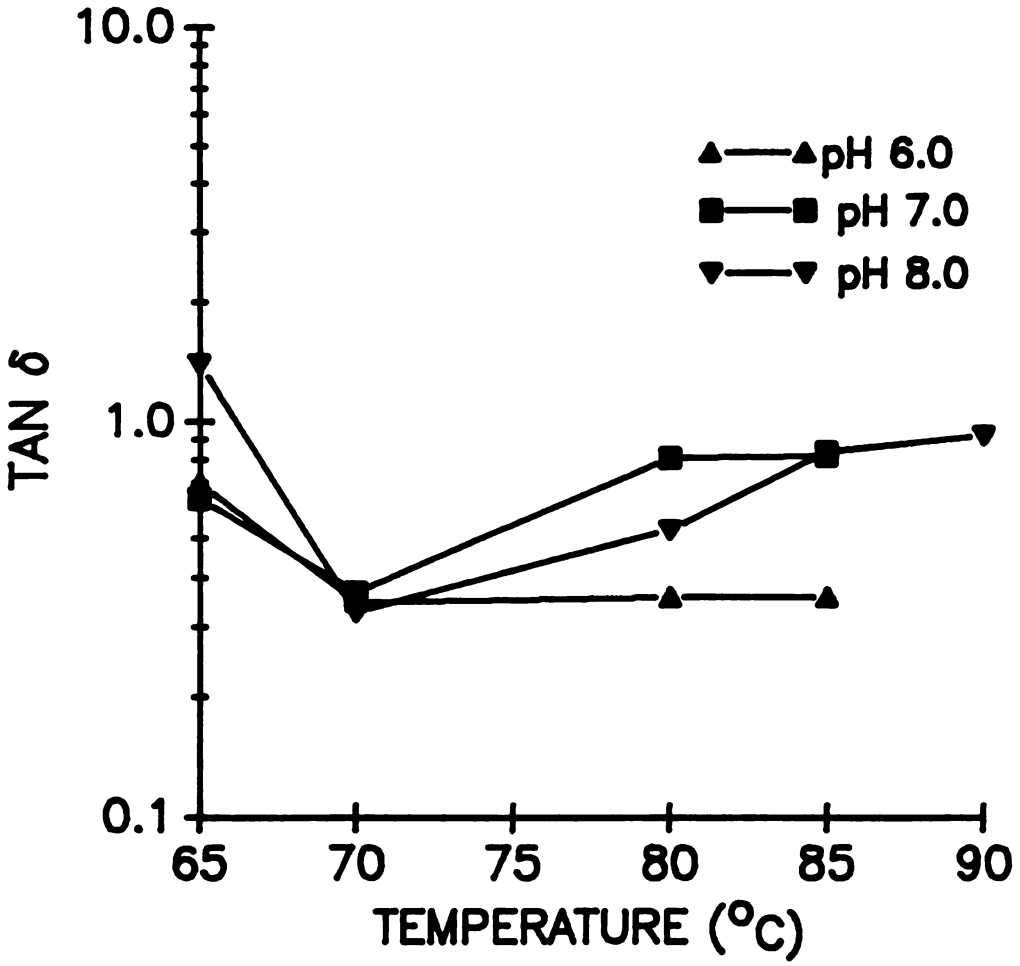

FIGURE 11. EFFECT OF pH ON TAN δ OF NAVY BEAN CONCENTRATE, 0.2M NaCl HEATED FROM 65–90°C AT 1°C/MIN

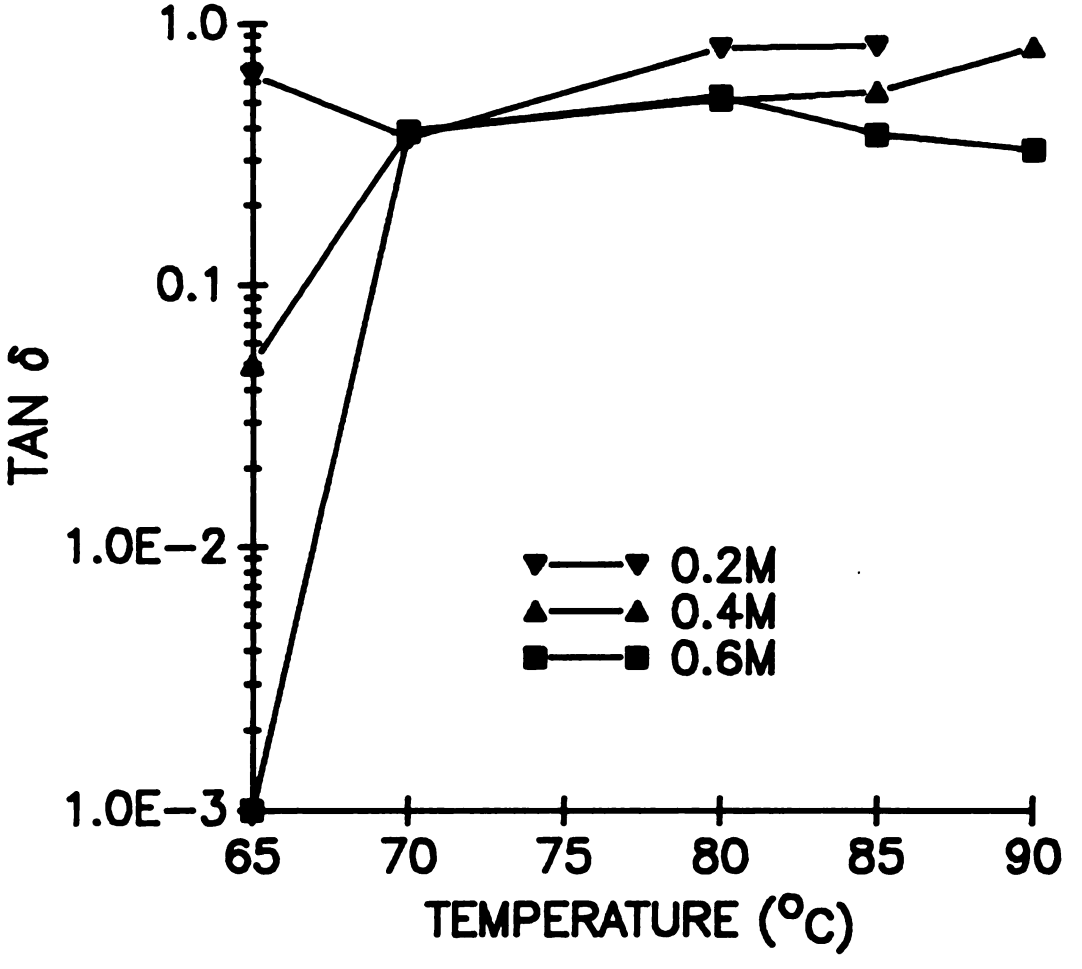

FIGURE 12. EFFECT OF NaCl ON TAN δ OF NAVY BEAN CONCENTRATE, pH 7.0 HEATED FROM 65–90°C AT 1°C/MIN

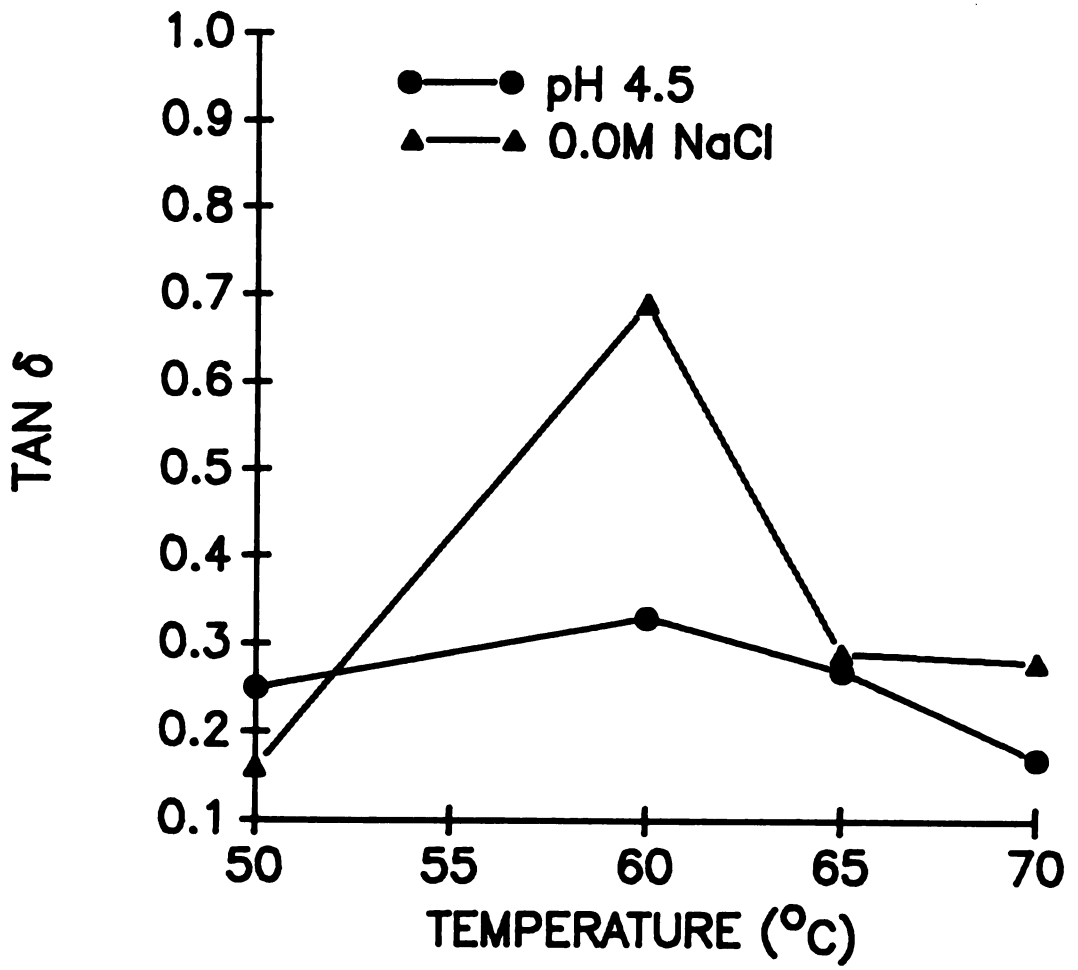

FIGURE 13. EFFECT OF HEATING NAVY PROTEIN CONCENTRATE AT pH 4.5 AND AT 0.0 M NaCl AT 1°C/MIN

Under these two conditions, a stable Tan δ was obtained almost immediately and remained stable until the tests ended at 90°C. The significant point to be made from Figures 11-13 is that the Tan δ value remains relatively constant throughout the entire process. This is probably due to two concurrent processes which take place during heating. The globular proteins begin to denature because of destruction of inter- and intra-molecular bonds which causes an increase in molecular diameter and a subsequent rise in elastic properties. Also, hydrophobic interactions are known to increase as proteins unfold so that formation of these bonds may contribute to the increase in elastic properties. Mechanical action of the instrument itself tends to break down gel structure causing an increase in the liquid characteristics of the solution.

Dejmek (1987) hypothesized that stable loss tangent values reflect formation and destruction of one type of bond. Thus, the stable loss tangent values may indicate that hydrophobic bonding is the major interaction involved in progel formation.

D. Conclusions

From these results several conclusions can be reached. It appears that the isoelectric precipitation procedure employed in this investigation tends to reduce

the amount of PHA present while at the same time rendering it more soluble.

The progel formation characteristics of a NPC are due primarily to the progel formation of the 7S vicilin fraction. SDS-PAGE indicated that this protein fraction is the largest component of both the soluble and insoluble protein NPC fractions. There are several minor fractions (including PHA) which in aggregate are a large percentage of the total protein content. Based upon DSC studies it would appear that these protein fractions tend to reduce the transition temperatures of progel formation. The transition reported in the DSC is approximately the point at which the progel formation of the navy concentrate as measured by the fluid spectrometer reaches a maximum.

The addition of NaCl appeared to stabilize the structure of the protein molecules from dry beans at higher NaCl concentrations. When no NaCl was added to the protein solution, the proteins aggregated instead of forming a progel. As NaCl concentration increased from 0.2M to 0.6M NaCl, progel formation was slower, indicating that the proteins were denatured less rapidly as the concentration increased.

The protein structure was apparently stabilized at pH 8.0 as compared to proteins heated at pH 6.0 or 7.0. This may be the result of component interactions which retard denaturation at this pH. Solutions at pH 6.0 and 7.0 had similar progel formation characteristics while the

solution heated at 4.5 (the isoelectric point) coagulated rather than gelled as shown by the quick increase in solid characteristics of the solution.

From this investigation, it would appear that the fluid spectrometer provides a good method for continuously following the progel formation of dry bean proteins. However, this method is best for comparing changes in solutions which are at the extremes such as 0.0M added NaCl and pH 4.5 and those at conditions which are normally seen in food systems. More subtle differences between solutions were not quite as readily seen, although this method is more definitive than more traditional analytical methods such as following the change in hydrophobicity or protein solubility as the solutions are heated.

VIII. Summary and Conclusions

A. Study I.

The results of Study I indicate that the lab scale air classification procedure will yield a large amount of high protein flour by remilling and reclassifying. Most of the losses experienced during the process can be minimized by reducing the amount of material lost during the initial milling steps. The second milling and air classification step with 050 mesh screen could be eliminated since the amount of high protein flour derived from this step is relatively small compared to the other steps.

The most satisfactory method for production of large amounts of a protein concentrate from dry bean flour is a modification of the isoelectric precipitation method of Fan and Sosulski (1974) in which the centrifugation speed is increased to 20,000 x g.

B. Study II.

The results of Study II indicate that the functional properties of the navy and kidney bean high protein flours and concentrates are generally comparable to those from soy beans. The high protein flours from dry beans produced gels with gel strengths significantly higher than the soy and the dry bean products had significantly higher soluble protein contents than the soy products. This was probably due to the defatting of the soy high protein

flour and concentrate and the spray drying of the concentrate. The high protein dry bean products also formed foams with significantly higher volumes than the soy bean products when whipped under high torque conditions. Conversely, the foams formed by the dry bean concentrates are less stable than those of the soy. This may be important when aqueous solutions of these products are accidentally subjected to high sheer and form undesirable foams. Processing of the dry bean meals into high protein flours and concentrates did not significantly change the protein digestibility while only the methionine content was significantly affected by concentrate production. While the protein products from dry beans may not entirely replace soy proteins, they might be better for use in certain applications.

C. Study III.

Several conclusions can be reached from the results of Study III. It appears that the isoelectric precipitation procedure employed in this investigation tends to reduce the amount of PHA present while at the same time rendering it more soluble.

The progel formation characteristics of a NPC are due primarily to the progel formation of the 7S vicilin fraction. SDS-PAGE indicated that this protein fraction is the largest component of both the soluble and insoluble protein NPC fractions. There are several minor fractions

(including PHA) which in aggregate are a large percentage of the total protein content. Based upon DSC studies it would appear that these protein fractions tend to reduce the transition temperatures of progel formation. The transition reported in the DSC is approximately the point at which the progel formation of the navy concentrate as measured by the fluid spectrometer reaches a maximum.

The addition of NaCl appeared to stabilize the structure of the protein molecules from dry beans at higher NaCl concentrations. When no NaCl was added to the protein solution, the proteins aggregated instead of forming a progel. As NaCl concentration increased from 0.2M to 0.6M NaCl, progel formation was slower, indicating that the proteins were denatured less rapidly as the concentration increased.

The protein structure was apparently stabilized at pH 8.0 as compared to proteins heated at pH 6.0 or 7.0. This may be the result of component interactions which retard denaturation at this pH. Solutions at pH 6.0 and 7.0 had similar progel formation characteristics while the solution heated at 4.5 (the isoelectric point) coagulated rather than gelled as shown by the quick increase in solid characteristics of the solution.

From this investigation, it would appear that the fluid spectrometer provides a good method for continuously following the progel formation of dry bean proteins. However, this method is best for comparing changes in

solutions which are at the extremes such as no added NaCl and pH 4.5 and those at conditions which are normally seen in food systems. More subtle differences between solutions were not quite as readily seen, although this method is more definitive than more traditional analytical methods such as following the change in hydrophobicity or protein solubility as the solutions are heated.

IX. RECOMMENDATIONS FOR FUTURE RESEARCH

Perhaps the most pressing need for research concerning the use of dry bean proteins in fabricated foods is for a systematic study of the proteins of the various varieties and their structure. Currently, information is extremely sketchy on the structure of the proteins and just how the proteins of similar bean varieties compare. The only protein which has had structures proposed for it is phytohemagglutinin which is used extensively for biochemical assays such as affinity chromatography. This however is a relatively minor constituent of the total bean protein and is known mainly for the many negative effects it has in the body. Dieckert and Dieckert (1985) concluded that there is very little disulfide bridging between the subunits of the vicilin and it has been shown that the inclusion of β-mercaptoethanol in the dissociating medium does not alter the size of the dissociation products (Derbyshire et al., 1976) How then are the proteins able to resist denaturation and aggregation for as long as they do at pHs close to neutrality? What is a good working model for the structure of dry bean proteins? Amino acid sequencing of the bean proteins would be helpful in this regard.

Another problem with the study of proteins from *Phaseolus vulgaris* is a simple lack of a data base upon which to build. In the study of these proteins and

their functionality in food systems, one is forced to use soybean proteins as a model because they have been extensively studied for a number of years and are very well defined. However, the proteins of the soybean may be different enough from those of dry beans to contribute to false conclusions about data from the bean proteins.

There is a great need for more basic studies of the bean proteins which are correlated with how they function in foods simply to gain a better understanding of these materials. Currently, it appears that the dry bean proteins have about the same limitations in food systems as soybeans. If these proteins are treated so as partially hydrolyze them for example, will their functionality improve? Can the fact that these proteins are so heat stable be taken advantage of in food systems?

Another issue which needs to be resolved is nomenclature. For example the major protein fraction in dry beans has been referred to as "glycoprotein II", vicilin, G-1 protein, and phaseolin, the 7S protein, and by the less adventurous "the major protein fraction". In these investigations, the author has used the nomenclature proposed by Bollini and Chrispeels (1978) simply for the sake of consistency.

Further study of the functionality of dry bean high protein flours and isoelectric precipitates should involve putting them into various food systems and investigating how their functionality compares with that of similar soy

products in the same food systems. If these dry bean high protein materials are ever going to be put onto the market, they will have to compete with soy which is not only cheaper but has a 30-35 year head start on the dry bean materials and thus is the material against which the dry beans should be compared.

It would have be interesting to correlate fluid spectrometer data taken on both a protein concentrate and purified vicilin, PHA, and legumin with DSC data to determine if indeed the transitions seen with DSC are actually the point at which gelation reaches a relative maximum. Is this really the point at which the most heat is absorbed by the proteins?

The results in this investigation should have been compared with those from a DSC under similar conditions. This was not done in this investigation because of time constraints. DSC data should have been able to confirm the results seen in the fluid spectrometer and possibly provide additional insight into the gelation process.

It would have been desirable to be able to follow the G* of the navy concentrate solutions during cooling as well as heating. This was impossible again because of fluid spectrometer limitations. In classical gelation theory, the cooling portion which is where the protein molecules actually form the covalent and ionic bonds which help form the structure of the gel. It has been shown by Van Kleef (1984) that the G′ of soybean isolate at pH 5.5

increases from 1 x 10^4 to 5 x 10^4. Damodaran (1988) theorized that the type of refolding which occurs during the cooling of 11S soy protein solutions was at least as important as denaturation of the protein during heating.

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APPENDICES

APPENDIX A

APPENDIX A

ANOVA Tables for Dry Bean High Protein Products Used in Study II.

(Note: * indicates significance at 5% level)

Table A1. Analysis of Variance (Protein)

Source of Variation	d.f.	s.s.	m.s.	F
Samples	5.00	729.64	145.93	34.88*
Error	12.00	50.20	4.18	
Total	17.00	779.84		

Table A2 Analysis of Variance (Moisture)

Source of Variation	d.f.	s.s.	m.s.	F
Samples	5.00	42.43	8.49	402.48*
Error	6.00	0.13	0.02	
Total	11.00	42.55		

Table A3 Analysis of Variance (Ash)

Source of Variation	d.f.	s.s.	m.s.	F
Samples	5.00	36.82	7.36	1018.11*
Error	6.00	0.04	0.01	
Total	11.00	36.86		

Table A9. Analysis of Variance (Foam Stability)

Source of Variation	d.f.	s.s.	m.s.	F
Samples	5.00	987	197	4.4*
Error	42.00	1887	45	
Total	47.00	2874		

Table A10. Analysis of Variance (Gelation)

Source of Variation	d.f.	s.s.	m.s.	F
Samples	5.00	217.16	43.43	8.32*
Error	18.00	93.93	5.22	
Total	23.00	311.09		

Table A11. Analysis of Variance (Soluble Protein)

Source of Variation	d.f.	s.s.	m.s.	F
Samples	5.00	2904.84	580.97	60.30*
Error	12.00	115.62	9.64	
Total	17.00	3020.46		

Table A12. Analysis of Variance (Digestible Protein)

Source of Variation	d.f.	s.s.	m.s.	F
Samples	5.00	217.16	43.43	8.32*
Error	18.00	93.93	5.22	
Total	23.00	191.87		

Table A13. Analysis of Variance (Methionine Content)

Source of Variation	d.f.	s.s.	m.s.	F
Samples	5.00	0.62	0.12	8.68*
Error	12.00	0.17	0.01	
Total	17.00	0.79		

APPENDIX B

APPENDIX B.

Plots Showing Effect of NaCl Concentration and pH on Solubility and Hydrophobicity of Navy Protein Concentrate

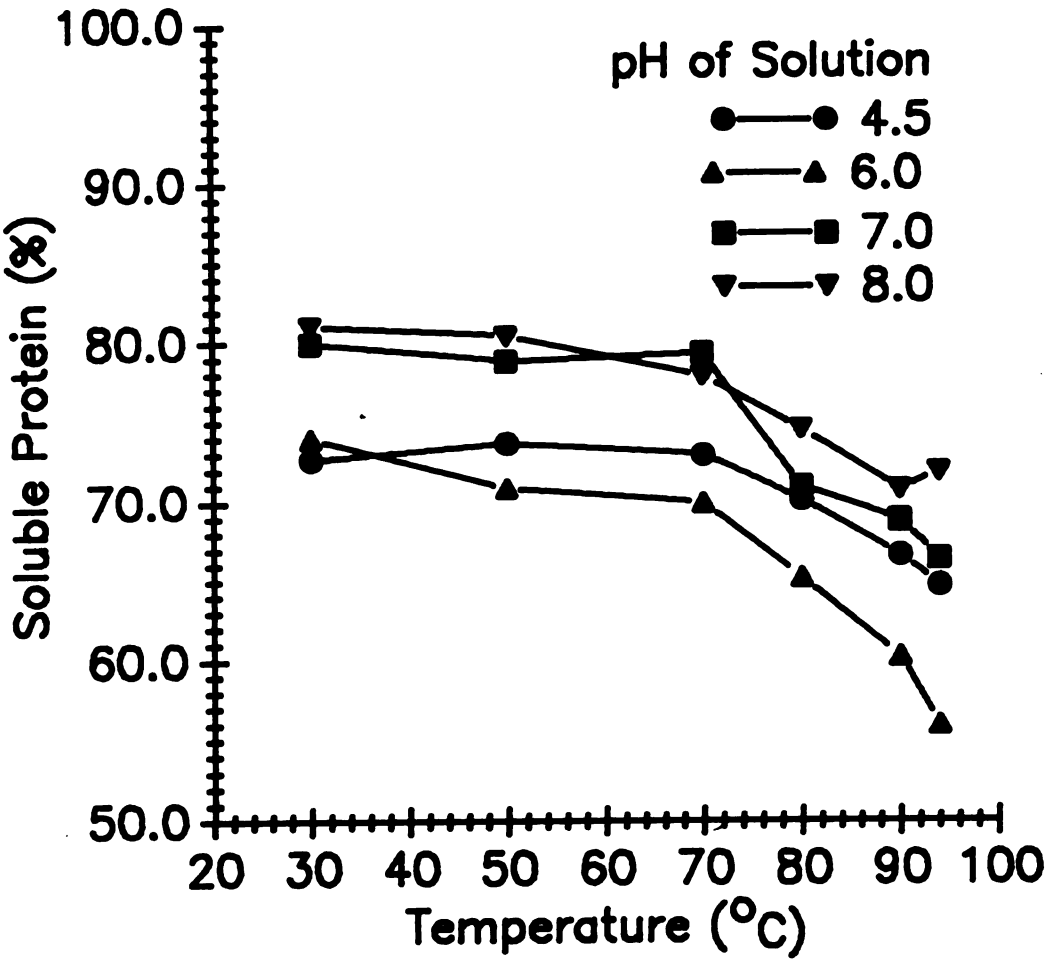

FIGURE B1. EFFECT OF pH ON PROTEIN SOLUBILITY OF NAVY BEAN CONCENTRATE, 0.2 M NaCl HEATED FROM 30–95°C at 1°C/MIN

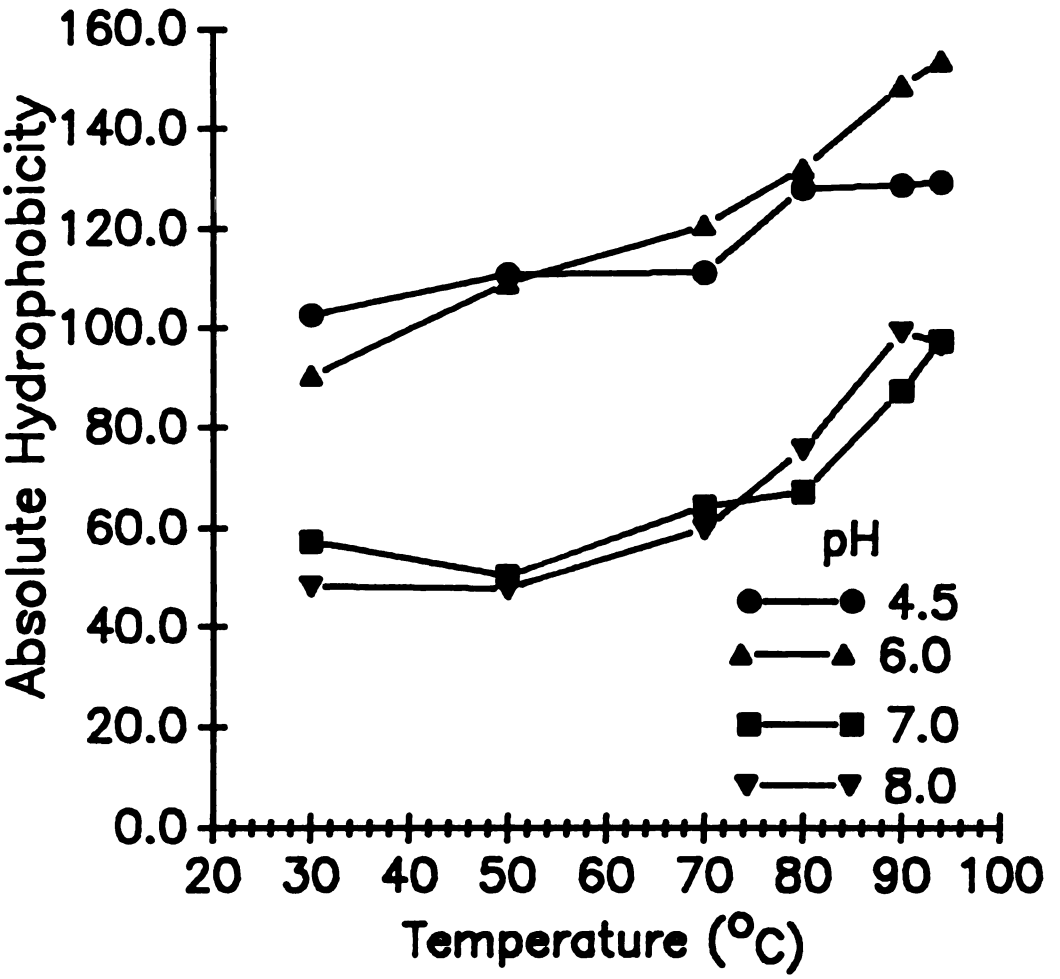

FIGURE B2. EFFECT OF pH ON HYDROPHOBICITY OF NAVY BEAN CONCENTRATE, 0.2m NaCl HEATED FROM 30–95°C AT 1°C/MIN

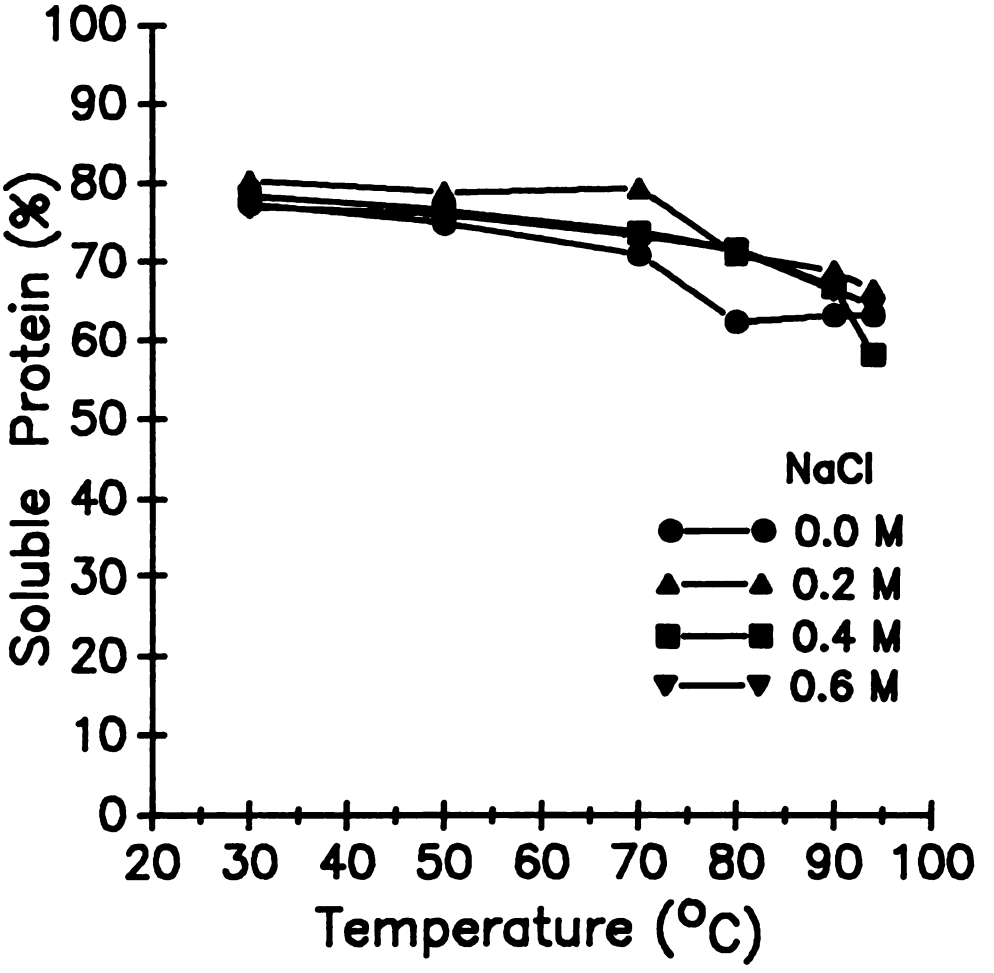

FIGURE B3. EFFECT OF NaCl ON SOLUBILITY OF NAVY BEAN CONCENTRATE, pH 7.0 HEATED FROM 30–95° AT 1°C/MIN

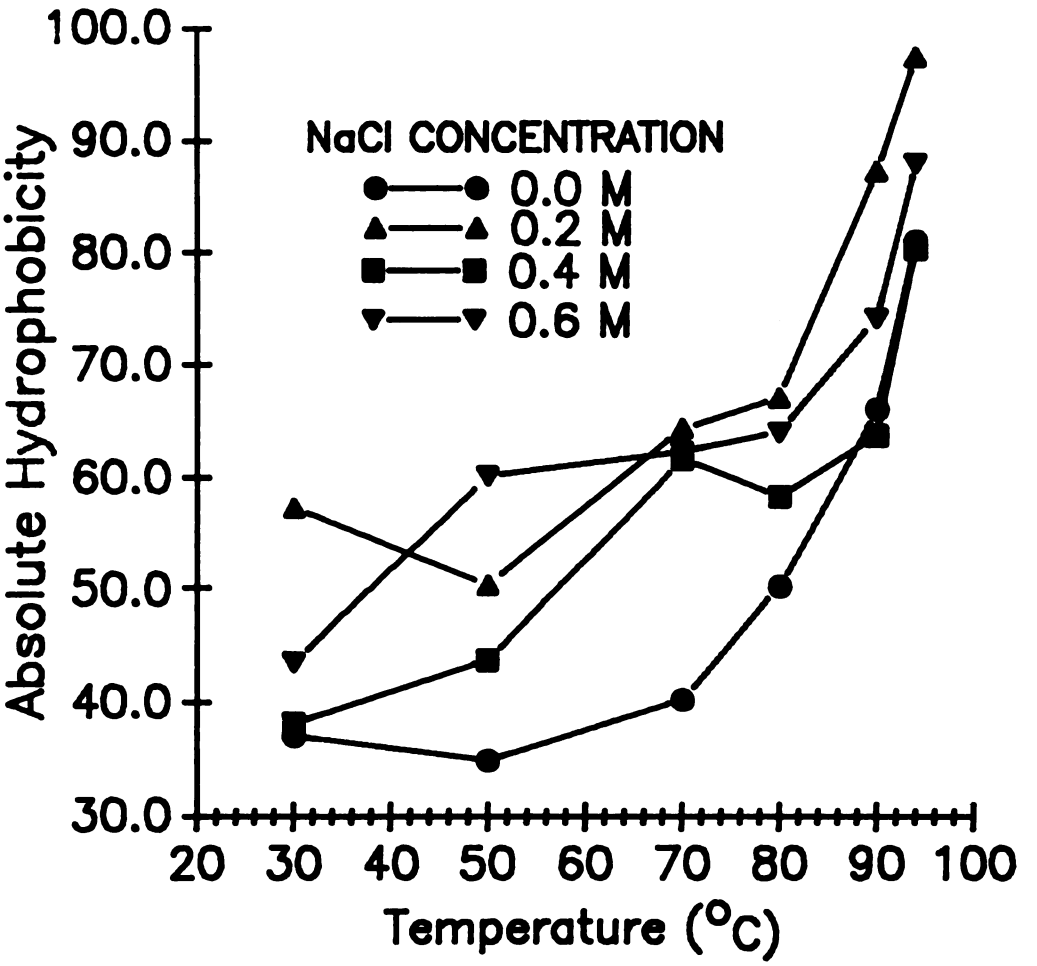

FIGURE B4. EFFECT OF NaCl ON HYDROPHOBICITY OF NAVY BEAN CONCENTRATE, pH 7.0 HEATED FROM 30–95°C AT 1°C/MIN