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COMPOSITIONAL, FUNCTIONAL AND NUTRITIONAL CHARACTERIZATION OF ULTRAFILTRATION PROCESSED COWPEA (Vigna unguiculata) AND NAVY BEAN (Phaseolus vulgaris) PROTEIN FRACTIONS

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JOSE CANDACE JACKSON

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COMPOSITIONAL, FUNCTIONAL AND NUTRITIONAL CHARACTERIZATION OF ULTRAFILTRATION PROCESSED COWPEA (*Vigna unguiculata*) AND NAVY BEAN (*Phaseolus vulgaris*) PROTEIN FRACTIONS

By

Jose Candace Jackson

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ABSTRACT

COMPOSITIONAL, FUNCTIONAL AND NUTRITIONAL CHARACTERIZATION OF ULTRAFILTRATION PROCESSED COWPEA (*Vigna unguiculata*) AND NAVY BEAN (*Phaseolus vulgaris*) PROTEIN FRACTIONS

By

Jose Candace Jackson

Ultrafiltration technology is increasingly being used to simultaneously purify, concentrate and fractionate macromolecules without the application of heat or the use of either extreme chemical or physical conditions. This is particularly important in isolating proteins since it results in very little modification of structure and functionality. Ultrafiltration is now a unit operation in the dairy industry. In the vegetable protein industry, most of the research has been limited to soy protein. There is thus tremendous potential to evaluate the effects of ultrafiltration processing on the physico-chemical properties of proteins from under-utilized leguminous species.

The optimum conditions for aqueous extraction of protein from milled cowpea and navy bean seeds was determined using particle sizes 0.79mm - 6.35mm, pH values 2-12 for 60 minutes each at 25 or 50°C. The legume flours were extracted three times and the extracts combined and pumped through a plate and frame ultrafiltration system. The flow rate (L/hr), flux ($L/m^2/hr$), protein content, recovery, molecular weight characterization and functional properties of freeze dried fractions were compared to a commercial soy protein isolate (SPI). The protein quality of the residue remaining after aqueous alkali extraction was determined. Legume and wheat flour diet blends were

made up to 10% protein and contained 30%, 70% and 100% by weight of the cowpea and navy bean residue supplemented with whole-wheat flour to 100%. Arrowroot starch was added as the major carbohydrate source. A 2% albumin diet was used to determine metabolic nitrogen, and a modified AIN-93G diet used as a control.

Alkaline pH was more effective than acidic pH in extracting protein. Particle size had a highly significant inverse relationship and temperature had no effect. Results indicate that the optimum protein extraction conditions were particle size of 1.59mm, at pH 10 and 25°C. Permeation flux ranged from 0.18 - 4.03 $L/m^2/hr$ and 3.12 – 24.80 $L/m^2/hr$ for cowpea and navy bean, respectively. Protein content of the fractions ranged from 18 - 53%. About 84% of the protein in the extract was recovered in the cowpea protein isolate (CPI), and 80% in navy bean isolate (NBI). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) indicated similar discrete band patterns for all protein fractions. The physico-chemical properties of cowpea and navy bean proteins were significantly affected by ultrafiltration (UF) processing, and there were significant differences between the experimental fractions and soy protein isolate (SPI). The protein quality of all experimental diets was significantly lower than that of the modified AIN-93G diet except the 30% cowpea diet. Diets with cowpea or navy bean as the primary source of protein were considered poor quality protein sources. The extraction and ultrafiltration system employed, provided discrete protein fractions that can be used as ingredients in the food industry; the 30%CP wheat flour diet blend could be recommended for use as food for pre-school children, ages 2 – 5 yr.

To my Mother - Evelyn

For your strength, courage and guidance

For always being there

Thank you

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Finally, but most importantly, my thanks for the spiritual guidance that I have received from my father, the Almighty God.

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INTRODUCTION

The word legume is derived from the latin "legumen" which means seeds harvested in pods. Alternative terminology for edible seeds of leguminous plants is "pulse" from the latin word "puls" meaning pottage. The term food legumes is used to cover both the immature pods and seeds as well as mature dry seeds used for human foods. According to the Food and Agriculture Organization (FAO, 1979), the word legume can be used for all leguminous plants. However for those containing small amounts of fat such as cowpeas and navy beans, the term "pulse" is used, and for those containing a high proportion of fat such as soybeans and peanuts, the term "leguminous oilseed" can be used (Salunkhe & Kadam, 1989).

Legume seeds have been an important component of human diet and an economical source of supplementary protein for many populations lacking animal protein for centuries, particularly so in developing countries. The average protein content of legumes (peas, chickpeas and dry beans) is about 22% compared with cereal crops such as rice and wheat with 7 and 12% respectively. Food legumes are however still under-utilized, primarily because of their prolonged cooking time requirements; deficiency of sulfur-amino acids; antinutritional components such as hemagglutinins (lectins), enzyme inhibitors, phytates, flatus factors, and tannins; as well as a low protein digestibility (Salunkhe & Kadam, 1989).

Sulfur amino acid deficiency, in particular methionine, has been well reported in the literature (Bressani & Elias, 1988; Deshpande & Damodaran, 1990, 1991; Bliss & Hall, 1977). On average, legumes contain 1 g methionine /100 g protein, the FAO

reference pattern suggests an intake of at least 2.2 g/100 g protein for optimal nutrition (Deshpande, 1992). Supplementation with the limiting amino acid has been recommended. Antinutritional components in legumes are of great importance since they can limit the nutritional potential for both human and animal consumption (Gatehouse, 1991). The two main types of antinutritional components in legumes include the proteinase inhibitors (primarily trypsin) and the lectins, and these have also been proven to be toxic in animal studies.

The use of ultrafiltration (UF) technology to separate macromolecules is a relatively new process in the food industry. It is an established unit operation in the chemical, environmental, and petroleum industries. In the food industry, the most important application is for concentrating proteins in dilute solutions, and most of the commercial applications involve the concentration of milk proteins and oil-seed proteins such as soybeans. Thus, there is tremendous potential for commercial concentration of proteins from under-utilized legumes such as cowpeas and navy beans.

The dissertation research has been divided into three studies as listed below:

1 Optimization of protein extraction from selected legumes

2 UF processing of aqueous alkali protein extracts into cowpea and navy bean protein fractions, and characterization and functional properties of the fractions compared to a commercial soy protein isolate

3 Evaluation of the nutritional properties of legume-wheat protein diet blends compared to a modified American Institute of Nutrition (AIN-93G) diet as the control

LITERATURE REVIEW

FOOD LEGUMES

Production, Distribution and Consumption

The major food legumes grown in all continents of the world include soybeans, groundnuts, dry beans, peas, broad beans, chickpeas and lentils. Others such as pigeon peas are grown only in some countries depending on the climatic conditions needed to support the growth and food habits of the population (Kadam & Salunkhe, 1989; FAO, 1990). The production of major legumes in developing and developed countries is indicated in Table 1. With the exception of soybeans and dry peas, production of legumes was significantly higher overall in developing countries than in developed countries.

World legume production, particularly of pulses, declined during the late 1970's to mid 1980's. This was due to the traditional caution of farmers which prevents them from allocating too high a proportion of their land to pulses because of low yields, uncertain harvests, slow maturation, and sensitivity of legumes to growing conditions at all periods of development and severe losses caused by pests. In addition, methods of preparation and cooking necessary to ensure a digestible product are often lengthy and costly in terms of fuel consumption. However, as research on legumes increased, production has steadily increased (Doughty & Walker, 1982; FAO Production Yearbook, 1996).

Food legumes are distributed throughout the world, probably because of their unique capacity to fix atmospheric nitrogen. Peas, broad beans, and lentils are in general more popular in the Middle East. Soybeans are consumed in large quantities in China,

Japan, Indonesia and Central America; while chickpeas and pigeon peas are popular in India, Bangladesh and Pakistan. Preference for one type of legume over another is determined by availability in that region, which is influenced by the environmental conditions that favor higher yields of certain species over another (Bressani, 1973; Kadam & Salunkhe, 1989).

Table 1 Production of Legumes in Metric Tons (MT x 10^3) in Developing and Developed Nations of the World [1]

Legume	Developed Nations		Developing Nations		Total (T)
	MT x 10^3	% T	MT x 10^3	% T	MT x 10^3
Dry Beans	2,206	12.5	15,376	87.5	17,582
Dry Broad Beans	415	12.0	3,031	88.0	3,458
Chick Peas	395	4.9	7,607	95.1	8,007
Cowpeas	56	2.2	2,502	97.8	2,560
Ground nuts	1,980	6.2	29,722	93.8	31,708
Lentils	545	18.6	2,390	81.4	2,954
Dry Peas	8,516	77.6	2,454	22.4	11,048
Pigeon Peas	---	---	2,787	100.0	2,787
Soybeans	69,054	52.9	61,551	47.1	130,658

[1] FAO, 1996

Dietary surveys indicate that bean consumption is very high in some countries, and that significant amounts of protein, calories and other nutrients are provided. However, there is a limitation to the amount of legume foods that are consumed by humans because they cause gastrointestinal disorders due to their bulk and low digestibility (Stanton et al, 1966; Kadam & Salunkhe, 1989).

Seed Structure

Food legumes are classified into two categories: those in which energy is stored as fat such as peanuts and soybeans; and those in which energy is stored as starch or gum such as cowpeas and navy beans (Doughty & Walker, 1982). However the seed structures in both types are similar. Generally, mature leguminous seeds have three major components: the seed coat, cotyledons and embryo axis.

The outermost layer of the seed is the testa or seed coat, and accounts for about 7.7% of the total dry weight in the mature seed (Powrie et al, 1960). The presence of polyphenolic compounds, primarily tannins, in varied amounts determines the seed coat color. The least amounts of tannins are shown in white beans like navy beans, and the amounts increase in the colored beans (black, read and brown beans) like cowpeas. In most legumes, the endosperm is only found at an early stage of development; in the mature seed, it is reduced to a thin layer surrounding the cotyledons or embryo. Characteristic external features include the hilium, micropyle, and raphe. The hilium is a large oval scar near the middle of the edge, where the seed breaks away from the stalk. The micropyle is a small opening in the seed coat beside the hilium and is the original site

where the pollen tube enters the valve. The raphe is a ridge at the side of the hilium opposite to the micropyle and represents the base of the stalk, which by maturity has fused with the seed coat (Doughty & Walker, 1982).

Bean seeds generally have a thick seed coat. The plumule or embryonic stem is fairly well developed in the resting seed and lies between two cotyledons or seed leaves. The radicle or embryonic root has almost no protection except for that provided by the seed coat. Thus, the seed is unusually vulnerable to breakage especially when dry and roughly treated (Doughty & Walker, 1982).

Studies have shown that the seed coat of many beans is made up of thick interwoven fibrous bundles, which is known as the cuticle. The hilium shows a great deal of variation in shape and size ranging from round to oblong and to oval and elliptical. The smaller seeds usually have a greater total surface area covered by the hilium. The micropyle varies from circular and triangular to fork shaped. The inside surface of the seed coat and the cotyledon surface have numerous hills and valleys, and appear to be complementary structures (Deshpande, 1985).

Chemical Composition

A wide variety of compositions exists for different legumes (Table 2), and this is governed by the cultivar, geographic location and growth conditions (Krober, 1968). They are characterized by a relatively large content of carbohydrates, ranging from 24-68%. The carbohydrates are either water-soluble components such as sugars and pectins; or insoluble fractions such as starch and cellulose. Protein content is generally

about 20 - 45%. Lipid content ranges from 1-7% except in the oilseeds such as soybeans and peanuts, which contain in some cases up to 50% lipids. Legumes are also good sources of dietary fiber and minerals, being a particular rich source of calcium, iron and water soluble vitamins such thiamin, riboflavin and nicotinic acid (Salunkhe & Kadam, 1989).

Table 2 Proximate Analyses (%)[1] and Nutritional Values of selected Leguminous seeds

Legume	H_20	Ash	Fiber	Fat	CHO	Protein	Nutritional Values	
							PER [2]	BV [3]
Blackgram	9.7	4.8	3.8	1.0	57.3	23.4	---	60-64
Chick Pea	9.8	2.7	3.9	5.3	61.2	17.1	1.7	52-78
Cowpea	11.0	3.6	3.9	1.3	56.8	23.4	---	45-72
Groundnut	5.0	3.7	2.4	48.2	15.9	24.8	---	---
Mungbean	9.7	4.0	3.3	1.2	58.2	23.6	2.1	39-66
Navy Beans[4]	12.6	3.2	9.6	1.2	63.3	23.4	---	---
Pigeon Pea	10.1	3.8	8.1	1.5	57.3	19.2	1.5	46-74

[1] Siegel & Fawcett, 1976
[2] Engel, 1978; and Luse & Rachie, 1979
[3] Bressani, 1973
[4] Uebersax and Occeña, 1991

Protein

The proteins of legume seeds are either metabolic, structural or storage in nature. The enzymatic (metabolic) and structural proteins are responsible for normal cellular activities including the synthesis of structural proteins and storage proteins. The storage proteins which are smaller in number, accounts for about 70% of the seed nitrogen (Moose & Pernollet, 1982), and occur within the cell in discrete protein bodies (Pernollet, 1978). The genotype and the environmental conditions under which the legumes were grown govern protein content. In most cases, variation between cultivars may be as much as 10-15%. The range of protein contents in various legumes is indicated in Table 3.

Table 3 Protein Contents (%) of Selected Food Legumes [1]

Legume	Protein Range
Chickpeas (Cicer arietinum)	14.9-29.6
Peas (Pisum sativum)	21.2-32.9
Faba beans (Vicia faba)	22.9-38.5
Cowpeas (Vigna unguiculata)	20.9-34.6
Winged beans (Psophocarpus tetragonolobus)	29.8-37.4
Pigeonpeas (Cajanus cajun)	18.8-28.5
Soybeans (Glycine max)	33.2-45.2
Lentils (Lens culinaris)	20.4-30.5

[1] Salunkhe & Kadam, 1985

The crude protein content based on the nitrogen determination of legumes involves the mixture of different nitrogen compounds. Along with proteins, there are free amino acids, amines, complex lipids, purine and pyrimidine bases, nucleic acids and alkaloids. These compounds are classified as non-protein nitrogen (NPN) compounds, and have been analyzed extensively in many legumes. The ratio of NPN to total seed nitrogen is in the range of 10 - 15%, and it is this which influences the factor that is used in calculating protein content for various protein sources (Earle & Jones, 1962; Gupta, 1982).

Proteins are located in the cotyledons and embryonic axis of beans with only a small amount being present in the seed coat (Singh et al, 1968). In navy bean seeds, the seed coat contains about 4.8% while the cotyledon and embryonic axis contains about 27.5% and 47.6% crude protein. The cotyledons because of their greater weight contribute the major amount of protein to the whole seeds. The outer cotyledon layer, which is about 60% by weight, was found to be richer in protein (Zimmerman et al, 1967).

Proteins have traditionally been classified as globulins, prolamins, albumins and glutelins based on their solubility (Osborne, 1907) properties. Globulins are soluble in dilute salt solutions, prolamins are soluble in 70% in ethyl alcohol, albumins are water soluble, and glutelins are soluble in diluted acids or bases. Storage proteins in most legume seeds are mainly composed of globulins, which acts as the carbon and nitrogen source during germination. These can be further sub-divided into phaseolin (vicilin), phaselin and conphaseolin (Bressani, 1975 and Kay, 1979). Phaseolin has been reported to have between three to five subunits ranging in size from 23KD to 56KD (Pusztai and Watt, 1970 and Derbyshire et al, 1976).

Studies on the amino acid composition of leguminous seeds, in particular S-amino acid deficiency (methionine), has been well reported in the literature (Bliss & Hall, 1977; Bressani & Elias, 1988; Deshpande & Damodaran, 1990, 1991). Legume proteins are mainly deficient in sulfur-containing amino acids (methionine) and tryptophan, but are rich in lysine, in which cereals are relatively deficient. In many agricultural systems throughout the world, legumes and cereals have been linked since they complement each other nutritionally. On average, legumes contain 1 g methionine/100 g protein, the FAO reference pattern suggests an intake of at least 2.2 g/100 g protein for optimal nutrition (Deshpande, 1992). Supplementation with the limiting amino acid has generally been recommended. The cotyledon as the major component of the seed accounts for 93% of methionine and tryptophan of the whole seed, while the seed coat is poorest in these amino acids. The embryo is rich in methionine and tryptophan but it contributes only about 2.5% of their total quantity in the seed (Kapoor & Gupta, 1977).

Carbohydrates

The total carbohydrates of dry legumes range from 24% in winged beans to about 68% in cowpea, including mono- and oligosaccharides, starch and other polysaccharides. Starch is the primary carbohydrate, and varies from 24% in wrinkled peas to 56.5% in pinto beans. Soybean, lupine and winged bean are reported to have the lowest starch content from 0.2 - 6.5%. The starch is believed to be embedded in a dense proteinaceous matrix, and the average size of the native bean starch granule ranges from 25 - 28 μm depending on the variety of bean (Kawamura et al, 1955).

Legume seeds are also reported to contain oligosaccharides such as raffinose, stachyose, and verbascose, the predominance of each type depends on the type of legume. Verbascose is the major oligosaccharide in broad beans and mung beans; whereas stachyose is found in peas, red kidney beans, cowpeas, soybeans and lentils. These oligosaccharides have reported to be involved in flatulence production in man and animals, although this is not harmful, it is a social discomfort to many people (Rockland et al, 1969; Levine, 1979; and Fleming, 1981). These sugars cannot be absorbed through the intestinal wall and cannot be digested by humans because the intestinal tract does not contain the enzyme - α-1,6-galactosidase that is required to split these oligosaccharides into simple sugars. These oligosaccharides pass through the gut and small bowel and enters the colon where bacteria readily utilize them as fermentation substrates and produce large amounts of carbon dioxide and hydrogen, and a small amount of methane (Levine, 1979).

The degree of flatulence appears to be related to the level of oligosaccharides in beans, as well as other non-oligosaccharide substances such as fiber (Reddy et al, 1984; Fleming et al, 1980). Legumes contain an appreciable amount of crude fiber, which consists of cellulose and hemicellulose. There is generally a large variation in fiber content between different legumes (Table 2). Cellulose is the main component of fiber in red kidney beans, navy beans, and cowpeas. In others such as lentils, broad beans and mungbeans, hemicellulose is the major component (Ali et al 1981).

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Lipids, Minerals and Vitamins

Lipid content is only of significance in the oilseed legumes such as soybeans and peanuts which contain about 20 and 40% lipids respectively; in general, the content ranges from 1-7%. In mature legumes, a major portion of lipids are stored in oil bodies or spherosomes or lipid containing vesicles in the cotyledons. Most legume lipids contain high amounts of essential fatty acids, the most important of which include linoleic and linolenic acids, which are required for growth, physiological functions and body maintenance (Doughty & Walker, 1982).

Food legumes are good sources of minerals such as calcium, iron, copper, zinc, potassium, and magnesium (Doughty & Walker, 1982). Potassium contributes about 25-30% of the total mineral content, and is useful in particular for the diets of people who take diuretics to control hypertension and who suffer from excessive excretion of potassium through body fluid. Calcium values vary widely, depending on variety, climate, cultural methods and mineral content of the soil. Legumes contain a significant amount of phosphorus which is largely present in the form of phytic acid, an antinutritional factor that affects the absorption and utilization of calcium through its precipitation as insoluble salts in the stomach and duodenum (Makower, 1969; Deshpande, 1992).

Food legumes are good sources of Vitamin B (thiamine and riboflavin), and niacin, but poor sources of Vitamin C (ascorbic acid) and Vitamin A (retinol) in the diet (Tapper & Ritchey, 1981). Studies have shown that the availability of Vitamin B_6 for intestinal absorption is reduced due to the presence of nondigestible polysaccharides and lignin (Gregory & Kirk, 1981). Legumes are also good sources of vitamin E (tocopherol) and folic acid.

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Antinutritional Factors

Antinutritional components in legumes are of great importance since they can limit the nutritional potential for both human and animal consumption. The two main types of antinutritional components in legumes include the lectins and the proteinase inhibitors (Table 4), and these have also been proven to be toxic in animal studies (Gatehouse, 1991).

Table 4 Lectin and Trypsin inhibitor activities of selected legume flours

Legume Flour	Lectin activity (units / mg dry matter)	Trypsin inhibitor activity (T.U.I. / mg dry matter)
Kidney Bean [1]	---	13.2
Cowpea [2]	< 0.05	21.1
Pea [2]	100 - 400	4.5 - 9.3
Faba Bean [2]	25 - 100	5.6 - 11.8
Soy bean [3]		
Raw flour	---	70
Defatted flour	1600 - 3200	85
Industrial meal (toasted)	25 - 200	0.63 - 5.5

[1] Valdebaouze et al, 1980
[2] Rouanet and Besançon, 1979
[3] Boulter, 1981

Phytohemagglutinins (PHA) or lectins were identified as one of the first factors involved in the toxicity of raw legumes to laboratory animals (Jaffe and Vega Lette, 1968; Pusztai and Palmer, 1977 and Wilson et al, 1980). Phytohemagglutinins (PHA) are tetrameric carbohydrate-binding proteins that exists as a mixture of five hybrids with

similar chemical properties but slightly different biological activities (Leavitt et al, 1977). The molecular weight of PHA of dry beans ranges from 115KD to 150KD, and the two subunits have molecular weights of 34KD and 36KD. Phytohemagglutinins (PHA) has potent biological activity because of its ability to bind complex carbohydrates and other glycoproteins. The adverse effects of PHA are the agglutination of erythrocytes and bacteria, binding to intestinal epithelium, and the stimulation of lymphocyte formation (Coffey, 1985).

It is believed that PHA releases intestinal amylase and lipase from binding sites on the glycocalyx of the intestinal epithelium, inhibits activity of intestinal saccaharase and brush border dipeptidases (Sandholm and Scott, 1979; Rouanet and Besancon, 1979; and Kim et al, 1976). Additionally, it has been shown that feeding pure PHA to rats' binds to and disrupts the intestinal epithelium leading to the formation of abnormal microvilli and damaged epithelial surfaces. This interference with intestinal surfaces and enzyme activity results in reduced protein digestibility and inhibits growth due to the depressant effect on appetite. The toxic effects, manifested as gastrointestinal discomfort, have also been reported in humans from a review of food poisonings in Britain. Seven outbreaks were attributed to food poisoning from kidney beans. In each case observed, the onset of symptoms of nausea, vomiting, diarrhea, and abdominal pain was rapid (1 - 3 hrs). Eating cooked and under-cooked beans were reportedly responsible (King et al, 1980).

Cooking beans has been reported to inactivate much of the lectin activity in dry beans. For complete elimination of the antinutritional effects, pre-soaking for at least 4 – 5 hrs, followed by heating either for 4 hr at 90°C, 90 min. at 95°C or 10 min. at 100°C is

required (RRI, 1982). Coffey et al (1985) reported on the lectin activity of low-temperature cooked kidney beans, and found that activity was still present in beans exposed to low temperatures for up to 12 hrs. This supported the work of Honavar et al, (1962) and Liener, (1958, 1962, 1976). They reported that lectin activity could be almost wholly eliminated by conventional heat treatments, however fully cooked beans can still contain a significant amount of the original lectin activity.

The proteinase inhibitors are substances that inhibit proteolytic enzyme activity and are specific in their interactions with proteinases such as serine proteases, sulphydrl proteases, metallo-carboxypeptidases and acid proteases, leading to pancreas hypertrophy. The growth depression caused by this inhibitor may be the consequence of an endogenous loss of essential amino acids being secreted by a hyperactive pancreas. Since pancreatic enzymes such as trypsin and chymotrypsin are rich in sulfur containing amino acids, this pancreatic hypertrophy causes the drain of body tissue with particular amino acids in order to meet an increased need for the synthesis of these enzymes (Gatehouse, 1991).

There have been a number of papers which described the heat stability/lability of these trypsin inhibitors (Estevez & Luh, 1985; Eicher & Satterlee, 1988; Esaka et al, 1987; Dhurandhar & Chang, 1990). It was concluded that the activity could be destroyed easily by 90% if the legumes were processed properly. This process involves at least 30-60 minutes in boiling water or autoclaving at 15 psi for 15-20 minutes (Deshpande, 1985).

Trypsin inhibitors fall into two main groups: those that have a molecular weight of about 20,000-25,000 D with relatively few disulfide bonds; and those with only 6,000-8,000 D and a high proportion of disulfide bonds (Liener, 1982; and Gatehouse, 1991). A

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study on trypsin inhibitors in pigeon peas by Godbole et al (1994) suggested that they were not associated with protein bodies. This suggested that the trypsin inhibitor could be removed from the legume without affecting storage protein structure and thus improves nutritional value of legumes.

Phytates, polyphenols, dietary fiber, and oxalates are dietary components in legumes that influence mineral bioavailability. Polyphenols such as the tannins have been ascribed certain beneficial effects such as lowering of blood-related disorders (Deshpande, 1985). Phytates interact with proteins, resulting in reduced protein solubility, and thus a reduction in solubility dependent functional properties of the protein, particularly if it is to be used as a food ingredient. Soaking of groundnut, pigeon peas, and chickpeas have been shown to lower phytate levels by leaching out the ions into soak water as a result of a concentration gradient (Igbedioh et al, 1994).

A number of studies have been conducted using purification and separation techniques such as ultrafiltration to remove antinutritional factors from legumes. In the study reported by Berót et al (1987), the authors found that α-galactosides, antitrypsic and hemagglutinating activity were lowered by submitting the protein extract to ultrafiltration. Air classification of fababean flour resulted in protein fractions that contained the larger proportion of trypsin inhibitors and lectins (Elkowicz & Sosulski, 1982); and alkaline extraction and isoelectric precipitation reduced the activity to about 1/3. The protein micellar mass (PMM) procedure described by Murray et al (1978, 1981) was reported to reduce the levels of antinutritional factors in the precipitated protein to less than about 5% of the original levels.

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Table 5

Legume
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[1] Boulter, J

[2] Gatehouse

[3] Martenss

[4] Thanh an

[5] Duranti e

LEGUME PROTEINS

The composition of proteins in selected leguminous seeds is presented in Table 5. It indicates that storage proteins in most legume seeds are mainly composed of globulins, which are about 90% in soybeans, and 60% and 66% for fababean and pea respectively. The globulins are made up of two subunits, which are characterized by their sedimentation coefficients. They are the 7S and 11S globulins and are called vicilin and legumin respectively. In soybeans and lupines, the oilseeds, the 7S-like protein is found in a larger proportion than the 11S-like protein; their ratios are 1.6:1 and 1.3:1 respectively (Duranti et al, 1981). In fababeans and peas, the pulses, legumin is the major protein, with the vicilin to legumin ratio being close to 1:2.

Table 5 Composition of proteins in selected leguminous seeds

Legume	Albumin [1] g /100g protein	Globulin [1] (g/100g protein)	Glutelin [1] g/100g protein	Vicilin: Legumin
Fababean	20	60	15	1:1.6 - 1:3.7 [2,3]
Pea	21	66	12	1:1.3 - 1:4.2 [2]
Soybean	10	90	0	1.6:1 [4]
Phaseolus	15	75	10	---
Lupinus	10 - 20	80 - 90	0	1.3:1 [5]

[1] Boulter, 1977

[2] Gatehouse et al, 1980

[3] Martensson, 1980

[4] Thanh and Shibasaki, 1976

[5] Duranti et al, 1981

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Protein Extraction

There are a number of protein extraction techniques that are available for use commercially for the production of food or food ingredients; or for purifying a protein from a food for further study in a laboratory. Generally, extraction techniques utilize the biochemical differences in protein solubility, size, charge, adsorption characteristics, and biological affinities for other molecules (Smith, 1994).

Dry processes for extracting proteins includes mainly air classification. In this method, light or fine protein fractions are separated from heavy or coarse starch fractions by milling to a fine powder, followed by several runs through an air classifier (Vose et al, 1976; and Tyler et al, 1981). Colonna et al (1980) reported on a two-run air classification process in which the protein fraction had a yield of 41% and 35% and a protein content of 68% and 56% for fababean and pea, respectively. Additionally, they found that legumes with higher lipid contents in the initial flour and a broad distribution of starch granule sizes such as wrinkled peas, tends to be less efficient when air classification is used for separating the protein from the starch.

Wet processes for protein extraction have been extensively reported in the literature (Anson and Pader, 1957; Flink and Christiansen, 1973; Murray, 1978; Murray et al, 1978, 1981; Berót et al, 1987; and Suelter, 1985). They are generally based on the differential solubility characteristics of proteins in solutions, which depend on the type and charge of amino acids in the molecule. Proteins can be precipitated or solubilized by changing buffer pH, ionic strength, dielectric constant or temperature (Smith, 1994).

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The most common is the isoelectric precipitation method that has been patented by Anson & Pader (1957). This involves alkali solubilization of the legume flour at pH = 7-10, followed by centrifugation to remove insoluble components, then isoelectric precipitation of the proteins at acid pH (3.5-4.5). Fan & Sosulki (1974) used this method to produce isolates from nine legume flours, and was able to show that the proteins were easily extractable in an alkaline solution and are less solubilized in the acid pH range around pH 4.

Proteins have been extracted with salt solutions. Satterlee et al (1975) produced a protein extract using 2% NaCl solution, followed by centrifugation at 9000 x g and dialysis of the supernatant for 48 hrs. Chang and Satterlee (1979) reported on a protein extract from 0.2% salt solution followed by precipitation at pH 4.0 at various temperatures. Sathe and Salunkhe (1981) fractionated bean flour using salt solubilization, dialysis, and finally freeze-drying. They were able to obtain a protein isolate and the isolated albumins and globulins from the bean protein. The protein content of the isolate was 92.43%.

Proteins can also be separated on the basis of their size due to the wide range of molecular weights (10KD to over 1000KD). Separation actually occurs based on the Stokes radius of the protein, and not on the molecular weight. Stokes radius is the average radius of the protein in solution and is determined by protein conformation. Actual extraction procedures that utilize separation by size include dialysis and ultrafiltration. Dialysis is used to separate molecules in solution by the use of semi-permeable membranes that permit passage of small molecules but not larger molecules. It is a simple process, but relatively slow that requires at least 12 hr. Ultrafiltration is similar to dialysis, in that it

uses a semi-permeable membrane, in this case however, separation occurs under an applied pressure and is much faster. Molecules larger than the membrane cut-off are retained and are called the retentate, while smaller molecules pass through the membrane and are called permeate (Kosikowski, 1986; and Smith, 1994).

Protein Functionality

Functional properties of proteins have been defined as those physico-chemical properties which give information on how a protein will behave in a food system (Hermansson, 1979). Functional proteins are important ingredients in the food industry; which is evidenced by the range of specialist ingredients traded and transported either as food protein groups or as individual proteins. For instance milk proteins are available as whole dried milk, casein or whey protein; egg white and wheat gluten are also available as individual proteins. Currently, there is now increased interest in new functional proteins, which has led to research on the functional properties of under-utilized protein sources such as legumes.

According to Sathe et al, (1984), protein functional properties can be classified into three major groups from a food application standpoint:

a) hydration properties are dependent on protein-water interactions, and encompass water absorption and retention, wettability, swelling, adhesion, dispersibility, solubility and viscosity;

b) properties related to protein-protein interactions including precipitation, gelation, and the formation of various other structures such as doughs; and

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c) surface properties such as surface tension, emulsification and foaming characteristics

Kinsella (1979) summarized the important functional properties of food proteins as organoleptic, hydration, surface, and structural/rheological. These properties reflect complex interactions between the composition, structure, conformation, and physico-chemical properties of the proteins, other food components, and the nature of the environment in which these are associated or measured. The factors that affect protein functional properties can generally be divided into three areas of influence: those that are intrinsic to the individual protein such as composition and conformation; and those processing treatments such as heating and pH; and environmental factors such as water, and temperature which affect the application of the protein (Kinsella, 1982).

The ability of proteins to bind and immobilize food components like water and lipids, is one of the most important functional properties in many food systems. This binding capacity is influenced by pH and ionic strength, and affects adhesion, film formation and viscosity (Kinsella, 1976). The amino acid composition, in particular, the polar amino acids, of the protein have a significant influence on protein-water interaction. Although they are the primary sites for protein-water interactions, electrostatic effects, as well as interaction between the amino acid residues themselves may also affect water binding by proteins (Perutz, 1978).

Protein solubility data is very useful for determining optimum conditions for the extraction and purification of proteins from natural sources, and for the separation of protein fractions. It has been reported that pH, temperature, processing conditions, and ionic strength affect solubility (Sathe & Salunkhe, 1981). Solubility behavior provides a

good index of the potential applications of proteins, because the degree of insolubility is the most practical measure of protein denaturation and aggregation. Solubility is also an important attribute of proteins selected for use in food beverages such as infant food formulas.

The viscosity of a fluid reflects its resistance to flow, and is expressed as the viscosity coefficient μ, which is the ratio of the shear stress (τ) to the relative site of shear or rate of flow (γ). That is, $\tau = \mu \gamma$. The main factor influencing the viscosity behavior of protein fluids is the apparent diameter of the dispersed molecules or particles. This diameter depends on the intrinsic characteristics of the protein molecule such as molar mass, size, structure, electric charges; protein-solvent interactions which influence solubility and swelling; and protein-protein interactions which determine the size of aggregates. Viscosity generally increases exponentially with protein concentration because of the high protein-protein interactions, and is an important functional property in fluid foods such as beverages, soups, sauces and creams (Damodaran, 1994).

Protein gels are defined as three-dimensional matrixes or networks of intertwined, partially associated polypeptides in which water is entrapped. The formation of gels is important in many foods including coagulated egg white, soybean tofu, and milk casein curd (Kinsella, 1976 and Schmidt, 1981). Damodaran (1988) has reported the stages that occur during heat-induced gelation. The initial step requires prior heating of the protein, which results in modification from the native state to a progel state. This involves dissociation and denaturation of the protein, which allows the functional groups involved in intra-molecular bonding to become available for inter-molecular bonding resulting in a gel network. Gel networks can be of two types. Those that contain high levels of non-

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polar residues and undergo random aggregation via hydrophobic interactions are opaque coagulum-type gels with low elasticity and water-holding capacity (i.e. irreversible gels). Reversible gels contain low levels of non-polar residues and is ordered, translucent, elastic, and has high water-holding capacity (Damodaran, 1988).

The protocol for testing gelling ability of proteins has been reported by Matsumura and Mori (1996). The first step involves solubilization or dispersion of various protein concentrations in neutral or weakly acidic conditions with gentle stirring. The protein solution is then heated in a boiling water bath for several hours and determination of the gelling point determined using dynamic rheology apparatus. The evaluation of gel properties is an important step in the process and this can be evaluated using scanning electron microscopy (SEM), spectroscopic analysis, gel solubility experiments, rheological measurements or measurements of fat-binding or water-holding capacity (Matsumura and Mori, 1996).

Rheological properties have been reported as the most important factors determining gel properties (Matsumura and Mori, 1996). The difficulty often lies in which rheological measurement to choose for the analysis. Deformation mechanical tests have been recommended such as texture profile, compression tests and tensile tests since they give rise to many parameters from which the required property can then be assessed. These parameters include hardness, cohesiveness, adhesiveness, stringiness, gumminess, chewiness, springiness and fracturability.

The behavior of proteins at interfaces influences the formation of food emulsions and foams. Food products that contain both water and fat form thermodynamically

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unstable mixtures or emulsions, which can then be stabilized with amphiphilic molecules or emulsifiers that are soluble in both water and non-polar solvents. Proteins are considered to be emulsifiers, and are capable of coating lipid droplets and providing an energy barrier to both particle association and phase separation during the formation of the emulsion. Many food emulsions are expected to remain shelf-stable for months, thus the process of emulsion stability or breakdown is important in the food industry. To be effective, the protein must have adequate solubility, must be flexible in order to reach the interface, unfold and orient itself with the hydrophilic groups towards the aqueous phase and the hydrophobic groups towards the lipid phase. The ability of the protein to lose its tertiary structure (denaturation) without loss in solubility is normally considered a positive attribute for an emulsifier (Mangino, 1994).

Studies on protein stabilized emulsions have been well reported in the literature (Kim and Kinsella, 1987; Halling, 1981; Swift et al, 1961; Regenstein, 1988; and Pearce and Kinsella, 1978). Measurement of emulsion capacity involves oil addition at a given rate to a defined quantity of protein dispersion until there is a decrease in viscosity or inversion. The change in viscosity is measured i) subjectively by visual appearance, ii) sudden drop in viscosity or iii) sudden increase in electrical resistance. This method has received considerable criticism since it requires an experienced operator, and also it measures the ability of proteins to form emulsions at protein-to-lipid ratios that are very different from what will be encountered in the finished product (Regenstein, 1988).

Measurements of emulsion stability mainly use the principle of oil and/or cream separation over a specified time period at a stated temperature. The causes of emulsion instability are coalescence, flocculation, gravitational creaming and Ostwald ripening, and

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most studies generally measure destabilization by coalescence and creaming (Petruccelli and Añón, 1994). It is possible to follow the formation of layers by the eye, by light scattering techniques, by confocal scanning light microscopy, and ultrasound (Blonk and Vanaalst, 1993 and Dagorn-Scaviner et al, 1987).

A foam is a complex two-phase colloidal systems containing a continuous liquid phase and a gas phase dispersed as bubbles or air cells (German and Phillips, 1994). Protein interactions in foams have been widely reported in the literature (Graham and Phillips, 1976; MacRitchie, 1978; Kinsella, 1981 and Kinsella and Phillips, 1989). For a protein to be a successful foaming agent it must be able to stabilize the surface area being created during foaming. It must be soluble and rapidly diffuse to the interface with the charged, polar and non-polar residues correctly distributed for enhanced interfacial interactions. The protein must be flexible to facilitate unfolding at the interface and reorient to form a viscous film to maintain discrete bubbles until stabilizing interactions develop (Prins, 1988; and Kinsella and Phillips, 1989).

Foaming capacity can be determined by three dynamic procedures: whipping, shaking or sparging (Waniska and Kinsella, 1979; and Yasumatsu et al, 1972). The major difference between these methods is the protein required; for whipping the amount ranges from 3 – 40%, 1% for shaking and from 0.01 – 2% for gas sparging (Yasumatsu et al, 1972). Once a protein foam is formed, its lifetime is dependent largely on the maintenance of the viscoelastic adsorbed layer. If surface denaturation occurs, it results in an insoluble coagulum and foam instability. Liquid drainage from the foam also contributes to foam stability.

Due to the transient stability of protein foams, they are often difficult structures to study. Direct measurement of the foam volume, bubble size distribution and lifetime provides a physical description of the foam. Indirect methods at the microscopic and molecular levels provides information about why one protein has better foaming properties than another does (Halling, 1981). Measuring the change in foam volume with time or the volume of liquid which drains from the foam are the most popular direct methods for assessing foam stability (Wang and Kinsella, 1976; and Graham and Phillips, 1976).

Nutritional Attributes of Legume Proteins

The primary function of dietary protein is to supply amino acids for the synthesis of body proteins and other nitrogen-containing substances. The body metabolism of proteins can be expressed by the difference between nitrogen intake and nitrogen elimination - the nitrogen balance. If this difference is positive, as occurs during growth, then nitrogen retention occurs through tissue deposition and protein synthesis. If it is negative as occurs with malnutrition, injury or infection however, then nitrogen is lost. In normal adults, the nitrogen balance is zero, because excess protein from large intakes are generally converted to energy and urea (Guthrie and Picciano, 1995).

Protein requirements in adults are assessed by measuring the minimal protein intake that will maintain nitrogen equilibrium, and in infants or children, by measuring the minimal protein intake that will provide an optimal rate of growth. The Food and Agriculture Organization and the World Health Organization of the United Nations

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(FAO/WHO, 1973) and the NAS (1980), have recommended "safe protein intakes" for healthy people which are in agreement with the results of N-balance experiments. In many countries, particularly those in the developing world however, the value recommended for safe protein intake is often overestimated to that which has been found necessary for a person to remain in nitrogen balance. In these countries, protein intake is habitually low, and the protein requirement is often lower, due to metabolic adaptation (UN University, 1979).

The protein nutritive value of a food corresponds to its ability to meet nitrogen and amino acid requirements of the consumer, and to ensure proper growth and maintenance. This is affected by the protein content, protein quality and the amino acid availability. Foods with protein contents below 3% such as cassava and arrowroot do not meet the protein requirements of humans even when ingested in large amounts more than the caloric requirements (Guthrie and Picciano, 1995).

The quality of a protein depends on the kinds and amounts of amino acids it contains, and represents a measure of the efficiency with which the body can utilize the protein. A balanced or high quality protein contains essential amino acids in sufficient ratios for human needs. Proteins of animal origin generally tend to be of higher quality than those of plant origin. Amino acids present in dietary proteins are not necessarily fully "available" since digestion of the protein or absorption of the amino acids may be incomplete. Amino acids from animal foods are absorbed to an extent of 90%, while those from plant foods are digested and absorbed to an extent of only 60-70%. This has been attributed to the protein conformation; binding to metals, lipids, cellulose and other polysaccharides; the presence of antinutritional factors such as trypsin inhibitors and

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lectins; as well as surface area and size of the protein, processing effects and biological differences amongst individuals (FAO, 1970, 1973).

Evaluation of proteins is useful to predict the amount or mixture of food proteins that would be necessary to meet amino acid requirements for growth and maintenance. In addition, it is also useful to rank the protein in terms of its potential nutritive value, and to allow the detection of changes in proteins after processing and storage (Pellett and Young, 1981; Bodwell et al, 1980; and Walker, 1983). Pellett (1978) reported that diet type (total protein, energy, amino acids etc), consumers (age, sex and their physiological status) and external factors such as food frequency, social, economic and hygienic conditions can affect protein utilization and hence protein quality.

The available methods for testing protein nutritional quality are shown in Table 6. Biological (*in-vivo*) assays measure growth or nitrogen balance as indicators of protein utilization and metabolism. These tests reflect the essential amino acid content, bioavailability of the amino acids present in the protein, and protein digestibility of the food being tested. Due to the time (30 - 50 days) and expense of biological assays, chemical or biochemical (*in-vitro*) assays have been developed to predict how a protein will meet nutritional and growth requirements. *In-vitro* assays include enzyme assays that model mammalian digestion for estimation of protein digestibility. Amino acid composition data is compared to reference proteins then corrected for digestibility (*in-vivo* or *in-vitro*) to obtain a protein quality estimate.

Table 6 Available methods for testing protein nutritional quality

Name of Assay	**Computation**	**Comments on Protein Quality (PQ)**
Growth and Nitrogen Balance		
Protein efficiency ratio (PER)	*PER = wt gain / protein consumed*	PER can underestimate PQ due to higher
	NPR = [wt gain of test group + wt loss of	

Table 6 Available methods for testing protein nutritional quality

Name of Assay	**Computation**	**Comments on Protein Quality (PQ)**
Growth and Nitrogen Balance Protein efficiency ratio (PER) Net protein ratio (NPR) Biological value (BV) Net protein utilization (NPU)	PER = wt gain / protein consumed NPR = [wt gain of test grp + wt loss of grp fed non-protein diet] / protein consumed BV = absorbed N from food – (urinary N – endogenous urinary N) NPU = [body N of test grp – body N of grp fed non-protein diet] / N consumed = BV x TD	PER can underestimate PQ due to higher need of rats for certain essential amino acids compared to humans, which grow at a much slower rate. PER also does not adequately account for proteins used in maintenance. NPR corrects PER value for maintenance BV and NPU are determined from N balance assays
Amino Acid Scoring Patterns Protein Digestibility Corrected Amino Acid Score (PDCAAS) Calculated PER (C-PER) Discriminate C-PER (DC-PER) Essential Amino Acid Index (EAAI)	PDCAAS = [mg of AA in 1 g test protein / mg of AA in 1 g reference protein] x PD C-PER = PER x digestibility DC-PER = Essential AA composition / FAO / WHO standard EAAI = [$\sqrt[9]{}$ (mg lysine in 1g test protein / mg lysine in 1 g reference protein) x (etc for all 8 essential AA + His)]	PDCAAS corrects for digestibility, and provides good estimate of protein quality C-PER and DC-PER consider the content of all essential amino acids, which is useful for foods with more than one essential amino acid in low amounts. Alternative to routine Q.C. screening of foods EAAI is useful as a rapid tool to evaluate PQ. Digestibility not included, therefore it would not account for differences due to processing or chemical reactions
Protein Digestibility *In-vivo* - Apparent Digestibility (AD) True Digestibility (TD)	AD = $[N_i - F_n / N_i] \times 100$ TD = $[N_i - (F_n - M_n) / N_i] \times 100$	TD is based on nitrogen ingested, feed intake, and corrected for metabolic losses in feces

Table 6 (cont'd)

Name of Assay	***Computation***	**Comments on Protein Quality (PQ)**
Protein Digestibility CTD In-VITRO - pH-Drop	*pH-Drop % D = 234.84 - 22.56 (X),*	pH-Drop is based on drop in pH with

Table 6 (cont'd).

Name of Assay	Computation	Comments on Protein Quality (PQ)
Protein Digestibility CTD		
In-vitro - pH-Drop	pH-Drop % D = 234.84 –22.56 (X), where X = pH at 20 min	pH-Drop is based on drop in pH with protein hydrolysis. pH not constant during assay. Does not accurately estimate differences between samples with low and high protein digestibility
pH-Stat	pH-Stat % D = 76.14 + 47.77 (B), where B = ml of 0.1 N NaOH added	pH-Stat assay is conducted at constant pH. More accurate than pH-Drop assay
Amino Acid Availability		
In-vivo (AA balance)	AA balance = AA intake (g) – AA excreted in feces (g)	AA balance can overestimate PQ because certain limiting essential AA are lost through microbial fermentation in the large intestine
In-vitro Microbiological		Microbiological assays are limited by the AA requirements of the microbe and certain food additives. In-vitro test is useful for ranking proteins as a result of processing
Available Lysine		
DNFB TNBS		DNFB is less suitable for partially hydrolyzed proteins or those with high reducing sugar. TNBS derivatives more susceptible to hydrolysis
Available Sulfur Amino Acids		
Met - CNBr and Me_2SO Cys - DTT & DTNB		Oxidized forms of Met not measured

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There have been numerous reviews on the nutritional quality of legume derived proteins and on the measurement of food protein digestibility (Bressani, 1975; Carpenter, 1984; and Satterlee et al, 1981). Bressani, 1973, showed that most of the legume proteins had low biological value, ranging from 32-78%. This has been attributed to the low concentration of sulfur amino acids in legume protein. Kelly (1973) showed the beneficial effects of the addition of methionine in the diet when legumes are used as the protein source. Additionally, Kelly (1973) found that both the protein efficiency ratio (PER), and the average weight increased. However, this was not evident for all legume species, and was explained on the basis that methionine is not the most or the only limiting amino acid in legume species. When both methionine and tryptophan were added, protein quality increased which indicated that both are equally limiting.

The protein efficiency ratio (PER) is the most common method that has been used to determine protein nutritive value. It is the weight in grams gained by rats per gram of protein consumed compared to that fed a control diet that contains casein as the sole source of protein. The method is based on the principle that the better the nutritional quality of the test protein, the more rapidly the animals will grow, and is generally reported relative to the casein control (Pellett, 1978).

This ratio depends on the protein consumed by rats, and protein quality can be overestimated for animal proteins and underestimated for some vegetable proteins. This is due to the higher need of rats for certain dietary essential amino acids compared to humans, which grow at a much slower rate. In particular, the PER method tends to overestimate the requirement for histidine, isoleucine, threonine, valine and sulfur-

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containing amino acids. PER also does not adequately account for proteins used in maintenance, and assigns a PER = 0 if the protein does not support growth (Rasco, 1994).

The net protein ratio (NPR) takes protein used for maintenance into consideration, and can be calculated as NPR = (Wt gain + wt loss of protein free group)/ protein ingested. Another approach calculates the net protein utilization (NPU) or the percentage of dietary nitrogen or protein that is retained. It is the product of the biological value (BV) - percentage of absorbed nitrogen retained in the body, and the coefficient of digestibility - the percentage of ingested nitrogen absorbed (NAS, 1980).

A number of protein quality tests utilize amino acid content data. Generally, the amino acid content of the test protein is compared to a reference protein, and protein quality is based upon the first limiting amino acid or all essential amino acids (Rasco, 1994). This amino acid score may be corrected for protein digestibility determined either by an *in-vitro* or *in-vivo* assay, which results in the protein digestibility-corrected amino acid score (PDCAAS) method, i.e. PDCAAS = amino acid score x % true digestibility. The amino acid score for each of the nine essential amino acids is the ratio of essential amino acid in 1 g of test protein to essential amino acid in 1 g of reference protein. Reference proteins rat growth and human pattern of amino acid requirements are indicated in Table 7.

When the amino score takes digestibility into consideration, it is believed to be a better estimate of protein quality since the body's utilization of dietary protein is affected by factors that are not reflected in an amino acid score. The PDCAAS method has been recommended by the FAO/WHO (1990) for measuring protein quality and has been

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adopted by the Food and Drug Administration (FDA) in their new nutritional labeling regulations for measuring protein quality of all foods except those intended for infants (FAO/WHO, 1990).

Table 7 Comparison of Suggested Patterns of Amino Acid Requirements for Humans[1] with that of the Rat[2]

Amino Acid	Amino Acids as % of Protein		
	Rat	Human	
		Pre-school Child (2-5 yr.)	Adult
Histidine	1.9	1.9	1.4
Isoleucine	4.1	2.8	4.0
Leucine	7.1	6.6	7.0
Lysine	6.1	5.8	5.5
Methionine + Cystine	6.5	2.5	3.5
Phenylalanine + Tyrosine	6.8	6.3	6.0
Threonine	4.1	3.4	4.0
Tryptophan	1.3	1.1	1.0
Valine	4.9	3.5	5.0
Arginine	2.9	---	---

[1] Rasco, 1994

[2] NRC, 1995

The calculated PER (C-PER) is a PER calculated from amino acid composition data of the test protein and *in-vitro* protein digestibility. The discriminate calculated PER (DC-PER) is calculated only from the essential amino acid composition of the food and compared to the FAO / WHO standard. C-PER and DC-PER methods consider the content of all essential amino acids unlike the amino acid score method (PDCAAS). This

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may be useful for foods that contain more than one limiting essential amino acid. The essential amino acid index (EAAI) is calculated using the ratio of the test protein to the reference protein for each of the eight essential amino acids plus histidine (Rasco, 1994). It is expressed as EAAI = [$\sqrt[9]{}$ (mg lysine in 1g test protein / mg lysine in 1 g reference protein) x (etc for all 8 essential amino acids + His)].

Protein digestibility assays provide important information about protein quality since all proteins are digested, absorbed and utilized to different extents. Differences in digestibility is directly related to the structure of the protein, the presence of non-protein dietary constituents (anti-nutritional components), processing and storage conditions, and modifications of the protein. Digestibility of the protein is the proportion of protein nitrogen that is absorbed, and is calculated based on the nitrogen ingested (N_i), feed intake, fecal nitrogen (F_n) and fecal metabolic losses of nitrogen (M_n). *In-vivo* assays determine the apparent (AD) and true digestibility (TD). AD =[(N_i - F_n) / N_i x 100] and TD = { [N_i - (F_n - M_n) / N_i] x 100}. *In-vitro* assays are calculated by either the pH-drop or pH-Stat methods using mammalian gastric and / or pancreatic and intestinal enzymes. The combinations of enzymes include pepsin, pepsin-pancreatin, papain, papain-trypsin, trypsin, trypsin-chymotrypsin-peptidase, and trypsin-chymotrypsin-peptidase-bacterial protease.

In-vitro assays measure the extent of hydrolysis or the initial rate of hydrolysis, and the constituents, pH, and temperature of the incubation medium are often fixed according to requirements of the enzyme reaction (Rasco, 1994). These assays however, do not take the amino acid balance of the food being test or fermentation of food proteins in the lower bowel into consideration.

The pH-Drop method described in AOAC Method 982.30 (1984) from studies by Hsu et al (1977) and Satterlee et al (1981) is based on the principle that a drop in pH occurs during protein hydrolysis. As the proteolytic enzymes break peptide bonds of the test protein, the freed carboxyl groups liberate a H^+ ion, which causes a decrease in pH. It has been reported that this method is sensitive enough to detect the presence of soybean trypsin inhibitors and to detect changes in digestibility due to processing (Rasco, 1994). The pH-Drop *in-vitro* method generally correlates well with data for *in-vivo* assays; however, they do not accurately estimate quantitative differences between samples with low and high protein digestibility. The major limitation of this method is that pH is not constant during the reaction and can be affected by other components in the food.

Pedersen and Eggum (1983) developed the pH-Stat method to overcome the problem in the pH-Drop method that pH is not constant. The two methods use similar enzymes, however in the pH-Stat method, digestibility is estimated from the volume of standard alkali (0.1 N NaOH) that is used to maintain a constant pH of 8.0 during hydrolysis. It has been reported that the pH-Stat is more accurate and gives better correlation with *in-vivo* assays. Pedersen and Eggum (1983) and Dimes et al (1994) reported correlation coefficients of 0.86 – 0.90 for pH-Stat vs. *in-vivo* digestibilities and 0.71 – 0.78 for pH-Drop vs. *in-vivo* digestibilities.

An *in-vitro* digestibility (Immobilized Digestive Enzyme Assay - IDEA) method has been reported by Chang et al (1990). Digestive tract enzymes are covalently immobilized on large-pore diameter (2000 A) glass beads via an amide linkage. The beads allow access of the test protein to the enzymes, and the method has been shown to correlate well with rat bioassays for a wide variety of food proteins.

Amino acid analysis is an important chemical technique for predicting protein quality. Spackman et al (1958) and Hill et al (1979) reported on the ion-exchange chromatography (IEC) and pre-column derivatization of acid hydrolyzates respectively. Both techniques resulted in complete destruction of tryptophan and partial destruction of the sulfur-amino acids during acid hydrolysis. These amino acids are often the limiting amino acids in the human diet, and separate hydrolyses of the protein are often recommended to determine their content. Thus for a complete analysis of all amino acids, at least three separate hydrolyses would be necessary: acid hydrolysis, performic acid oxidation followed by acid hydrolysis for sulfur amino acids, and methane sulfonic acid (MSA) hydroysis for tryptophan.

In-vitro and *in-vivo* amino acid availability methods measure the relative digestibilities of the individual amino acids. The *in-vivo* assay referred to as the amino acid balance is calculated as the difference in weight between the amino acid intake and the amino acid excreted in fecal matter. This method can overestimate protein quality because it does not account for the loss of some limiting essential amino acids by microbial fermentation in the large intestine. The *in-vitro* assay is particularly useful in studies that evaluate the effect of processing treatments on protein quality.

The available lysine is usually tested in protein quality assays because the free amino group on the side chain of lysine can react chemically with other food components during processing and storage to give complexes that are biologically unavailable. Reactive lysine (free ϵ-amino group) can be measured directly using reagents such as 1-fluoro-2, 4-dinitrobenzene (DNFB), trinitrobenzenesulfonic acid (TNBS), ω-

methylisourea, or σ-phthalaldehyde. Indirect methods include DNFB difference method, dye-binding procedure, furosine method, or reduction by $NaBH_4$ (Rasco, 1994).

Available sulfur-containing amino acids should also be determined in protein quality analyses since they are often the limiting amino acids in foods and can be readily oxidized to forms that are no longer bioavailable. Rasco (1994) reported several methods to determine available sulfur-containing amino acids. Available cysteine / cystine can be measured by converting cystine to cysteine with dithiothreitol, reacting cysteine with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) and measuring the quantity of the derivatized form. Methionine can reduce dimethyl sulfoxide (Me_2S), which can be quantified by headspace gas chromatography, or it can also react with cyanogen bromide (CNBr) and the reaction product methylthiocyanate (MeSCN) measured by gas chromatography as an indication of available methionine.

MEMBRANE SEPARATIONS

The primary role of a membrane is to act as a selective barrier, permitting the passage of certain components and retaining other components of a mixture. This implies that either the permeate or retentate phases should be enriched in one or more components (Cheryan, 1986). A membrane has been defined as "a region of discontinuity interposed between two phases", or as "a phase that acts as a barrier to prevent mass movement but allows restricted and/or regulated passage of one or more species through it" (Lakshminarayanaiah, 1969).

The basic concept of a membrane separation is shown in Figure 1. A feed stream generally enters the membrane system and a driving force, such as pressure or concentration, is applied across the membrane effecting the separation. Certain components such as solutes, gases or solvents, can pass through the membrane, while others do not pass through, or only very slowly. This selective transport is the basis of membrane separations. The stream that is able to traverse the membrane is referred to as the permeate or filtrate, while that which is retained is called the retentate or concentrate (Mohr et al, 1989).

The flux is the rate that the permeate passes through the membrane, and is usually expressed in units of volume of permeate per unit time per unit membrane area (flux units gal/ft^2/day, GFD; or L/m^2/hr LMH). The rejection is a measure of the membrane's ability to separate or retain solution components. It is the fraction of the components that is retained by the membrane, and is generally expressed as a percentage.

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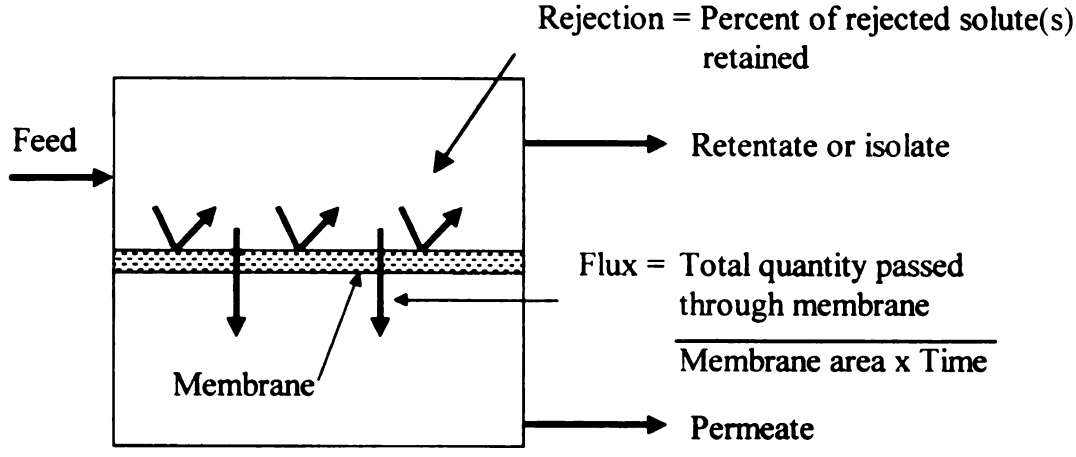

Figure 1 Membrane Concepts (Mohr et al, 1989)

Mathematically, it can be expressed as:

$$\text{Rejection} = 100\ \frac{(F_i - P_i)}{F_i}$$

where i = specific component or group of components

F = concentration of i in the feed stream

P = concentration of i in the permeate stream

Recovery is the fraction of the feed that is recovered as permeate, and permeability is the rate at which components permeate through the membrane. It is generally expressed in units of quantity times membrane thickness per unit time per unit membrane area per unit of driving force (Cheryan, 1986; NFPA, 1993).

The five major membrane separation processes include reverse osmosis, ultrafiltration, microfiltration, dialysis and electrodialysis; and are capable of separations over a wide range of particle sizes. Centrifugal separations are also versatile, however it requires that there be a density difference between the two phases that are to be separated. This is not so for membrane separations, and their usefulness lies in that they permit separation of dissolved molecules down to the ionic range provided an appropriate membrane is used (Cheryan, 1986).

Membranes, their components and characteristics have been extensively described by Cheryan, 1986; Mohr et al, 1989; and NFPA, 1993. They are made up of various materials, either inorganic or organic; and may be isotropic or anisotropic. Isotropic membranes have a similar texture and pore structure from one side of the membrane to the other, while anisotropic membranes change structure from the surface to the interior of the membrane.

The factors that are important considerations for membrane selection include separation capabilities, i.e. retention or selectivity; separation rate or flux; chemical and mechanical stability of the membrane; and the membrane material cost. Desirable membrane properties are high flux and selectivity, chemical resistance, and durability. Flux and selectivity depend on the membrane material, while chemical resistance and durability are affected by the entire membrane system including membrane, housing, adhesive materials etc.

The organic membranes are the most common commercially, and include those made from cellulose acetate (CA), polysulfones (PS), and polyamides (PA). CA is utilized mostly for RO and some UF membranes, their use in food processing is restricted because

they can only tolerate a narrow pH range (3-8) and a maximum temperature range of 35-40°C. They are also subject to degradation from organic solvents, and microbial activity; and are sensitive to chlorine. Aromatic PS membranes are used in UF systems, and are more suited for food processing. They can tolerate temperatures up to 150°C; and are resistant to low levels of chorine and other oxidizing agents and solvents; and can tolerate a fairly wide pH range (~ 2-14). The aromatic PA's are common in RO systems, and to a lesser extent UF and MF. They are generally resistant to chemicals, higher temperatures, and pH; but are sensitive to chlorine and other oxidizing agents

The inorganic membranes are more suited to food applications than the organic membranes because they can tolerate harsher conditions in terms of temperature (up to 300°C), chemicals, pH (1 - 14), and chlorine tolerance. They are used only in UF and MF applications. Materials for inorganic membranes include glass, sintered metal, ceramics, and inorganic polymers.

Membrane Module and System Structures

Membrane separation equipment or modules have been reviewed by (Cheryan, 1986; Mohr et al, 1989; and Ho & Sirkar, 1992). They comprise of four basic types: flat plate, spiral-wound, tubular and hollow-fiber. The type of module being utilized depends on the type of separation, throughput/cost, process flexibility required, ease of maintenance, and ease of operation. The flat plate or plate-and-frame module was one of the earliest systems, and can be used with any membrane that is available in a flat sheet. It consists of a series of membranes sandwiched between spacers that act as flow channels,

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and which results in a high packing density. The membranes are bonded to an inert support material, which is porous and offers little resistance to flow. This module can accommodate low levels of suspended solids and viscous fluids due to the thin feed flow channels, which provide high fluid turbulence at lower pumping rates. It can also be used to test, and monitor different membranes. The membrane packing density is somewhat low and thus requires more space, initial capital cost is high, membrane replacement labor is high and the modules are very susceptible to fouling due to the thin channels.

Spiral wound modules are an extension of the flat plate systems. They comprise of two layers of membrane, generally sandwiched around a porous, woven permeate carrier or spacer. Three sides of this sandwich are sealed, and the fourth is open and attached to a perforated tube. A feed channel carrier or spacer is layered over the membrane envelope, and all is wound spirally around the central perforated tube or collection pipe. The feed flows axially into the channels, and permeate penetrates the membrane and travels up the permeate carrier spirally to the central tube (Cheryan, 1986; NFPA, 1993).

The spiral wound system has a higher membrane packing density, and this minimizes the space needed for the system. It generally requires a lower capital because of the less expensive materials used in manufacturing. Its disadvantages includes its inability to handle suspended solids, the solids tend to plug the mesh of the feed channel spacer; this makes cleaning difficult if particles get caught up in the feed channel spacer. In addition, the use of plastics in the permeate carrier and feed channel spacers limits their use at higher temperatures due to deformation which can occur at high temperature and pressure (Cheryan, 1986; NFPA, 1993).

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Tubular modules resemble the structure used in heat exchangers. The diameter of the tubes range from 1/8" up to about 1"; and the modules are able to handle suspended solids with little or no pretreatment, and higher viscosity fluids. Feed solution is introduced at one end of the tube and it flows through the tube, while the permeate passes through the membrane. Permeate is collected in an outer shell, and the retentate exits at the other end of the tube. The larger the tube, the higher the pumping rate needed to minimize buildup of the boundary layer on the interior of the tube (Cheryan, 1986; NFPA, 1993).

These tubular systems are operated under turbulent flow, which minimizes buildup of solids on the membrane. They are relatively easy to clean by CIP (clean-in-place) techniques; the diameter allows dirtier water with suspended solids and fluid foods to be processed; and if one tube fails it can be plugged off and the rest of the system allowed to run. The disadvantages of these modules are their lower membrane packing density, which requires more floor space, the high capital cost, and their higher pumping energy costs due to large feed channels, large pressure drops and high flow rates. In addition, there is usually a high hold up volume per unit of membrane area (Cheryan, 1986; NFPA, 1993).

Hollow fiber modules consist of hollow, hair-like fibers with an outside diameter of 50μm to about 1mm, which are bundled together into either a U-shaped or a straight-through configuration. In the U shaped or closed end modules, a loop of fibers is inside a pressure vessel, feed enters the shell, is pressurized, and permeate passes through to the center of the hollow fibers. The permeate exits the open fiber ends at the same end of the pressure vessel that the feed entered. The remaining fluid exits the module at the end

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opposite the feed entrance. These fibers tend to be smaller in diameter, and cannot handle suspended solids (Cheryan, 1986; NFPA, 1993).

In the straight-through modules, fibers are placed inside a pressure vessel and are open at both ends. The feed fluid is circulated on the inside of the fibers with permeate passing through to the outside of the fibers. To minimize pressure drops, these fibers have somewhat larger diameters than those used in the U-shaped module, which allows them to accommodate low levels of suspended solids. This type of module is used for ultrafiltration.

Hollow fiber modules have very high packing density; this requires less floor space and thus has lower capital cost. They can be back-flushed for cleaning, and low-pressure drops and flow rates contribute to lower energy costs. However, the modules are delicate, and can be easily damaged during use. The hollow fine fibers are highly susceptible to fouling by suspended solids build-up, and cleaning can be difficult because there is little space between the fibers. Further to this, if a capillary fiber is damaged, the entire module must be replaced (Cheryan, 1986; NFPA, 1993).

During membrane processing, fouling of the membrane can occur, which results in a decline in flux with time of operation. Considerable research has been conducted to describe the fouling process, and design conditions to reduce fouling of membranes, particularly whey protein fouling of membranes (Blatt et al, 1970; Parkin, 1975; Lee, 1977; Smith and MacBean, 1978; Merin and Cheryan, 1980; Luss, 1985a, 1985b; Kim, 1989 and Ramachandra et al 1994).

Fouling occurs primarily because of a deposition of matter (either by adsorption, precipitation, pore plugging, particulate adhesion, chemical reaction or other interactions)

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within the filtration device or within the internal pore structure of the membrane or onto the membrane surface. Foulants can include dissolved organic matter such as proteins or carbohydrates; microorganisms; inorganic compounds such as carbonates, or sulfates; and colloidal or particulate matter such as suspended solids or metal oxides.

Cleaning can generally restore fouled membranes completely or partially. In some cases however, when the fouling leads to changes in the membrane structure such as with compaction or creep (change in membrane which decreases its permeability and flux) the fouling leads to permanent change and is irreversible (NFPA, 1993).

Ultrafiltration Processing

Ultrafiltration (UF) is a relatively new process utilized in the Food Industry to separate molecules through a porous polymeric membrane on the basis of their molecular weight. It results in concentration of higher molecular weight solutes in the concentrate, and the production of a lower molecular weight permeate stream. UF membranes generally rejects solutes in the size range of 1.0 to 100 nanometers or molecular weight of 300-500 to 300,000-500,000 Dalton depending on the molecular weight cutoff (MWCO) of the membrane (NFPA, 1993).

Under normal conditions of transport through a semi-permeable membrane, water flow occurs from the dilute to the more concentrated solution, a process referred to as osmosis. The pressure that causes this type of flow is called the osmotic pressure. If however a pressure is applied to the concentrated solution that exceeds the osmotic pressure, then the flow direction is reversed, and water migrates from the more

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concentrated to the dilute solution (Lewis, 1982; Cheryan, 1986). This results in further concentration of the more concentrated solution. Thus the pressure to cause this opposing flow direction must be high enough to exceed the osmotic pressure of the solution and to overcome the hydraulic resistance of the membrane.

UF is used for the separation of fairly large molecules, such as natural polymers like proteins, starch, and gums, and colloidally dispersed compounds such as clays, paints, pigments, latex particles etc.; hence the osmotic pressures involved in this separation process are relatively low. This means that UF has the advantage of having lower equipment and operating (pumping) costs by a considerable margin. In addition, it does not require any complicated heat transfer or heat-generating equipment since only electrical energy is needed to drive the pump motor. UF processes can be operated at ambient temperatures even though at times it may be necessary to operate at low temperatures to prevent microbial growth problems or denaturation of heat sensitive components; or high temperatures to lower viscosity of the retentate or to improve mass transfer. UF is however limited in the sense that it cannot take the solutes to dryness which is due to the low mass transfer rates obtained with concentrated macromolecules, and the high viscosity that makes pumping of the retentate difficult (Cheryan, 1986).

Due to the fact that UF membrane systems retains macromolecules or particles larger than about 0.001-0.02 μm, they have found use as a technique for simultaneously purifying, concentrating and fractionating macromolecules without the application of heat or the use of either extreme chemical or physical conditions. This is extremely important in protein work since it results in very little modification of structure and functionality (Lewis, 1982).

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UF is now a well accepted processing operation in the dairy industry. It is used in the fractionation of cheese whey and pre-concentration of milk for cheese manufacture, and is well reported in the literature (Chapman et al, 1974; Abbot et al, 1979; DeBoer and Nooy, 1980; Bush et al, 1983; Garoutte, 1983; Barbano and Bynum, 1984; Cheryan and Chiang, 1984; Cheryan and Kuo 1984; Ernstrom and Anis, 1985; Ernstrom, 1986).

Vegetable protein products have been a part of the diet of many Asian and African countries for centuries, but it was not until recently that the western world recognized their food value. Soybeans, now the major source of edible oil in the United States, has received considerable research as a potential protein source. Lawhon et al (1981, 1982) reported on the use of a combined aqueous extraction and membrane isolation process to obtain a single isolation procedure to produce protein and oil food products from un-defatted soybeans. They also used UF membranes to co-process soy protein extracts and skim milk to produce a soymilk food ingredient. A number of authors have also studied the removal of antinutritional factors from soybeans using UF membranes, and improving functional properties of soy protein isolates (Cheryan, 1979, 1988; Hartman, 1979; Omosaiye & Cheryan, 1979; and Lah & Cheryan 1980).

More recently, research has begun to be expended on other leguminous species, as their desirability as less expensive protein sources becomes obvious, particularly to nations where protein-energy malnutrition was prevalent. Ko et al, (1994) reported on the use of UF to recover protein from simulated waste water during mung bean starch preparation, and found that protein recovery was about 87.8% using a 30,000 MWCO membrane, which increased the utilization of mung bean, and reduced pollution problems. Ulloa et al (1988) obtained a protein concentrate from chickpea (*Cicer arietinum*) by UF membranes

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and used it in infant formulas. Berot et al, (1987) studied ultrafiltration of fababean protein extracts and found that yields were higher at alkaline pH, and the isolate had better functional properties such as whipping and thickening than standard isolates.

MATERIALS AND METHODS

Materials

Mature dry seeds of cowpea (*Vigna unguiculata* cv. California Blackeye No. 5) and navy beans (*Phaseolus vulgaris* cv. Huron) grown in the 1995 crop year were obtained from Bayside Best Beans LLC, Sebewaing, MI. These seeds were stored in a walk-in cooler in the Food Processing Laboratory in the Department of Food Science and Human Nutrition at Michigan State University at 4°C (39°F) prior to use.

Proximate Analysis

Cowpea and navy bean seeds were milled in a Udy Cyclone Mill (Udy Co., Fort Collins, CO), and all other samples (extracts and ultrafiltration fractions) freeze dried prior to proximate analyses.

The moisture content was determined using AACC Method 44-40 (1984). Protein content was determined using the micro-kjeldahl Method 46-13 (AACC, 1984), and the conversion factor of 5.7 used for legume proteins, and 6.25 used for casein and albumin. Fat was determined using AACC Method 30-25 (1984), and ash by AACC Method 08-01 (1984). Carbohydrate was calculated by difference, that is, subtracting the components determined above from the total solids (TS) as determined from moisture content.

Milling and Laboratory Scale Protein Extraction

An overview of the protein extraction procedure is shown in Figure 2. The seeds were milled in a Fitzpatrick mill (Model D Comminuting Machine, Fitzpatrick Co., Chicago, IL) to pass through five different screen sizes (0.79-6.35mm). The bean flour (3

g) was dispersed in 24 ml of distilled water and the pH adjusted to between 2 and 12 with 1N acetic acid or 1N sodium hydroxide (NaOH). The mixture was shaken in a 25°C or 50°C water bath for 60 minutes. The extracts were separated from the residue by centrifugation (8800 g, 20 min), then nitrogen content determined on the extract by the micro-Kjeldahl method (AACC, 1984). The protein content was calculated using the nitrogen to protein factor of 5.7. Soluble solids (%) of the extracts and protein yield expressed as g protein / 100 g flour were also determined. The flours were then extracted sequentially three times using the optimal conditions and protein content and yield determined.

Pilot Scale Protein Extraction

A schematic process for the pilot plant extraction of cowpea and navy bean flour is indicated in Figure 3. The seeds were milled in a Fitzpatrick mill (Model D Comminuting Machine, Fitzpatrick Co., Chicago, IL) to sieve size 1.59 mm. A series of extractor baskets (200 mesh) was used in the extraction process to separate the residue from extract. 20 L of distilled water and 2 Kg of bean flour was added to the extractor basket, and the pH adjusted to 10 with 400 ml 1N sodium hydroxide (NaOH). The mixture was re-circulated for 60 minutes at 25°C, to ensure complete extraction, then passed through a series of cheesecloth to separate any fibrous matter that passed through the basket. The flour was re-extracted twice under the same conditions until there were three extracts produced. The three extracts were combined, then insoluble components removed after overnight sedimentation (4°C).

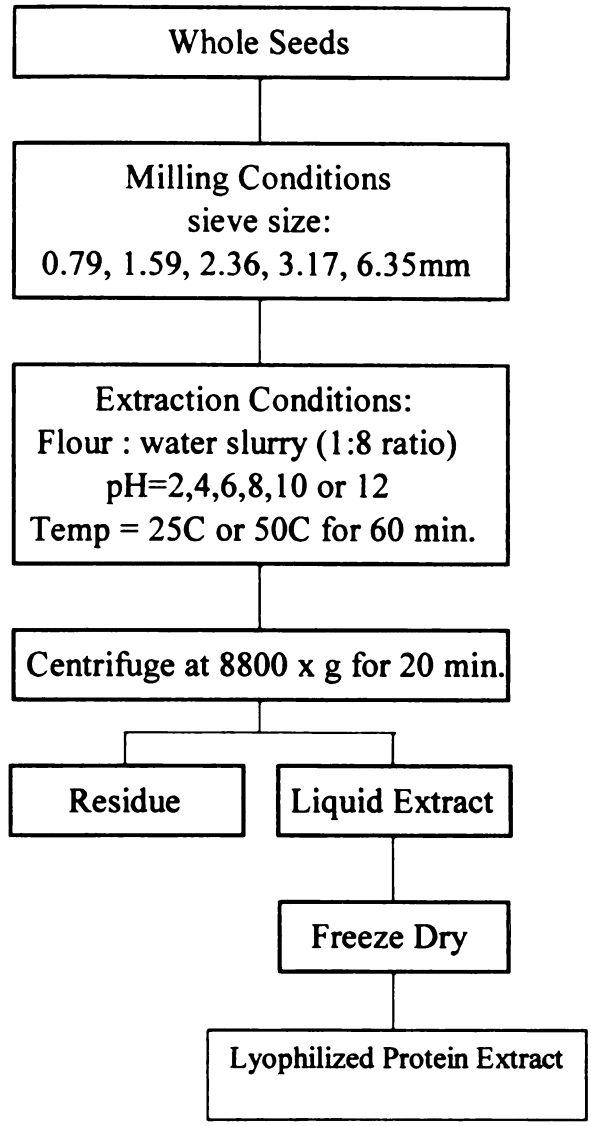

Figure 2 Schematic of Laboratory Scale Protein Extraction from Cowpea and Navy Bean Flour

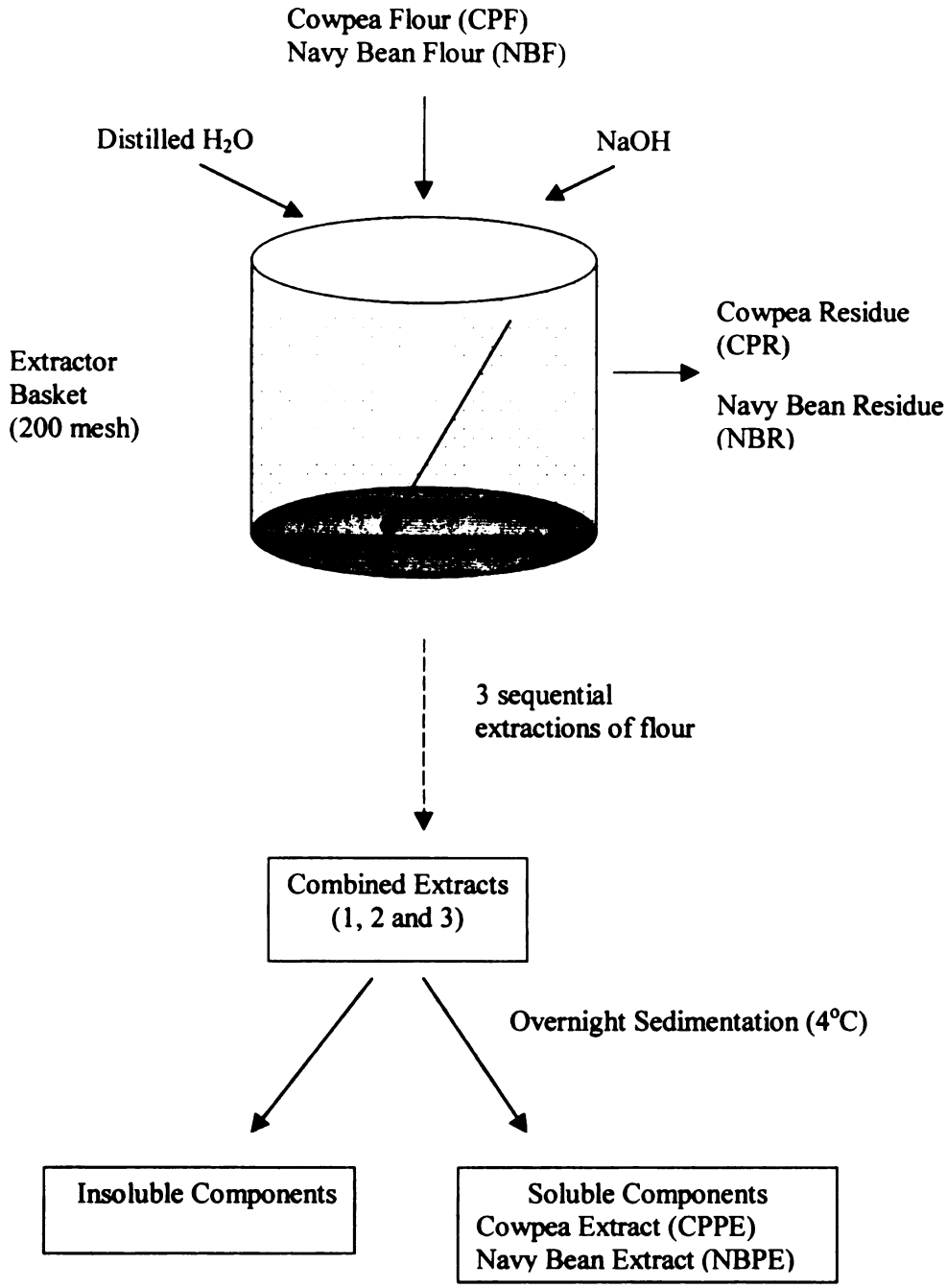

Figure 3 Schematic of Pilot Plant Scale Extraction of Cowpea and Navy Bean Flour

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Ultrafiltration Processing of Aqueous Extracts

The soluble protein extracts were poured into a 114-L holding vat. A positive displacement pump was used to transfer the extract to the Ultrafiltration Lab Module, Type 20 system (Dow Danmark A/S Separation Systems, Denmark) where it passed through a pre-filter (80 micron) and then into a large feed stock vat. The extract was pumped from the feed stock vat and passed through Desal (Osmonics Inc., California) HG19, HN28 and HZ20 flat membranes in series (Figure 4). The molecular weight cut off (MWCO) points for the membranes were 5-10KD, 15-30KD and 50-100KD respectively, and the total surface area was $0.863m^2$. The average transmembrane pressure was set at 3.4 atm, and pump speed 3, to maximize flow.

The volume of permeates and retentate collected in timed intervals from 0 – 7 hr using a graduated cylinder and stopwatch was measured, and the total weight of each fraction determined at the end of the run. The flow rates [volume / time = L/hr] per batch and permeation rates or flux [volume (L) / membrane area (m^2) / time (hr) = LMH] were calculated.

After each run, the membranes were cleaned using a caustic (Ultrasil 10 & Ultrasil 25) and acid (Ultrasil 75) UF cleaning program for proteins (Figure 5). They were then soaked in a 0.25% metabisulfite solution until the next use (EcoLab, St. Paul, MN).

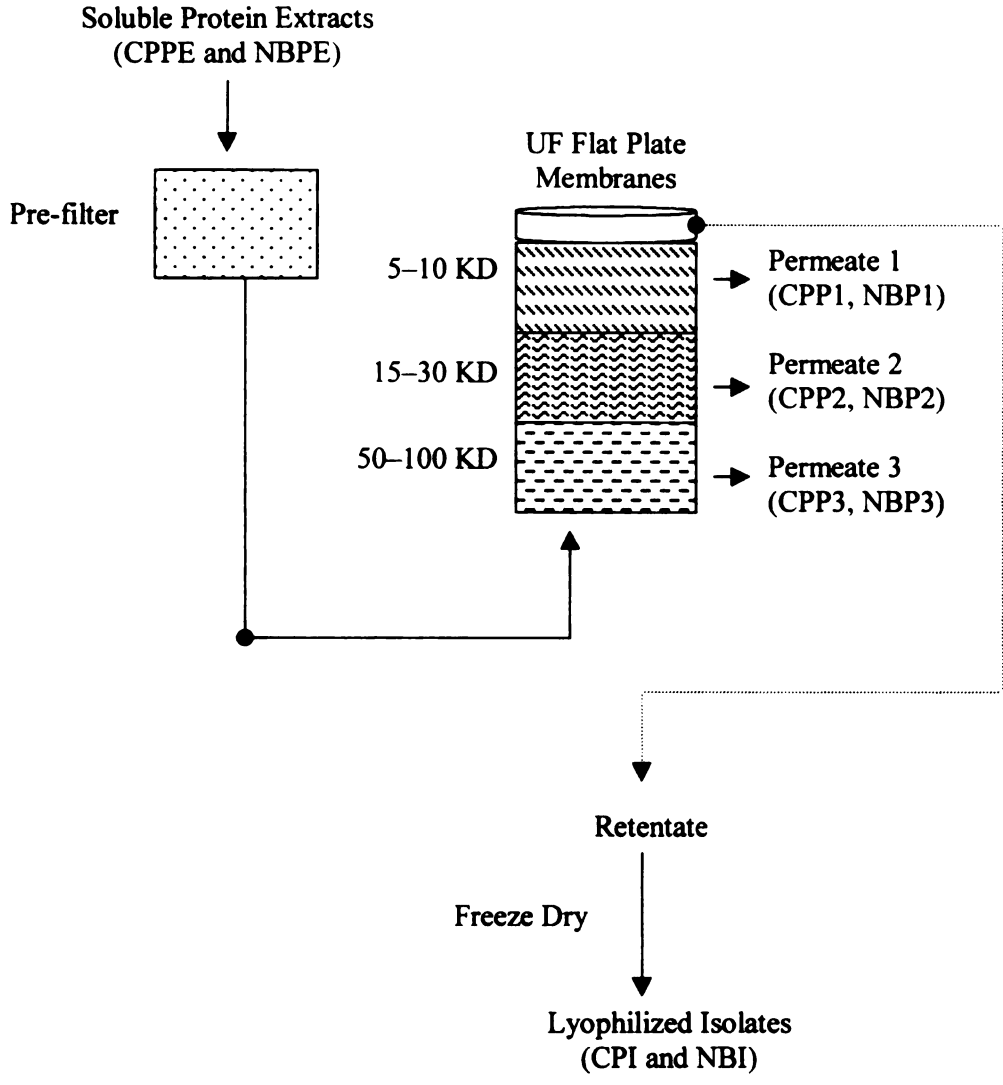

Figure 4 Flow diagram of Ultrafiltration Processing of Cowpea and Navy Bean Aqueous Alkali Extracts

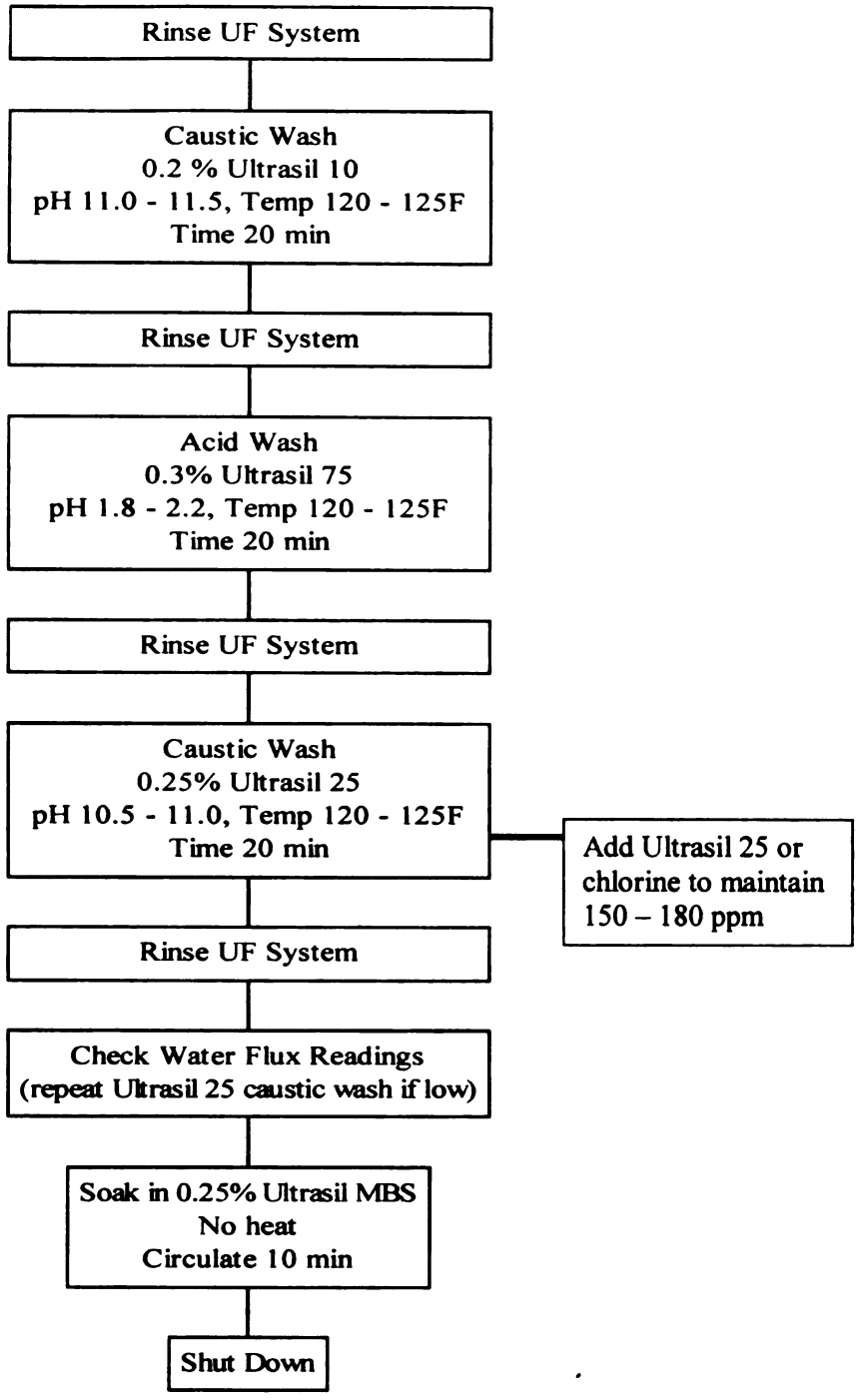

Figure 5 Ultrafiltration Processing Cleaning Program recommended for Protein Products, (EcoLab, 1992)

SDS Polyacrylamide Gel Electrophoresis

A modification of the SDS PAGE method of Ng (1970) was used. Solutions for 10% running gel and 7% stacking gel were prepared as shown in Table 8. The running gel solutions were mixed in a beaker and the reagents for polymerization (APS and Temed) added. The resulting solution was mixed briefly and the gel poured. The gel was covered with a thin layer of water or alcohol and allowed to stand for at least one hour. After polymerization, the top of the gel was rinsed with water and the plates turned upside down to remove excess water. The reagents for the stacking gel were mixed and stirred similarly. The gel was poured to completely fill the plate and the comb inserted; it was then allowed to stand for at least one hour. After polymerization of the stacking gel, the combs were removed, and the sample slots rinsed with water then filled with electrode buffer.

Sigma wide molecular weight range (M-4038) protein marker (15µl) was loaded at each end of the gel, and sample (10–25 µl) loaded in the slots between. Electrophoresis was carried out with a Hoeffer Vertical Electrophoresis unit Model SE 600 (Hoeffer Scientific Instruments, San Francisco, CA) using a constant voltage power supply (Fisher Biotech Electrophoresis System, Model FB 458, Pittsburgh, PA). A constant current of 25mA was applied for 24 hrs until the dye front reached the bottom of the gel. After the run, the plates were opened and the gels placed in staining solution for 24 hours. The gels were destained in several washes of deionized water, then a second staining was done in Blakesley solution (Table 9) for 24 hours (Ng, 1970). The gel was then rinsed in deionized water, and photographed. Sub-unit molecular weights of the protein fractions were estimated using the Sigma protein markers.

Table 8 Preparation of Running and Stacking Gels for SDS PAGE Analysis

Reagents	Volume (ml)	
	10% Running Gel (30 ml)	7% Stacking Gel (10 ml)
30% acrylamide	10	2.50
1.5% bis-acrylamide	2.60	1.10
Tris-HCl	12	4
Water	4.35	2.29
10% sodium dodecyl sulfate (SDS)	0.30	0.10
1% ammonium persulfate (APS)	0.75	0.25
N,N,N',N'tetramethyl-ethylenediamine(Temed)	0.02	0.0067

Table 9 Preparation of Blakesley Solution for SDS PAGE Analysis

Reagent	Quantity
Coomassie Blue G90	2 g in 1 L H_2O
2 N H_2SO_4	55 ml in 945 ml H_2O
10 N KOH	123.4 g up to 220 ml with distilled H_2O
100% TCA	300 g in 300 ml H_2O

Thermal Stability

The values of onset (T_i) and peak (T_p) temperatures (in ^oC) and enthalpy change (ΔH in W/g) for the protein test samples were studied using differential scanning calorimetry (DSC). The magnitudes of T_i and T_p were determined as the intersection between the baseline and the tangent to the descending segment of the endotherm and the minimum point on the curve, respectively. ΔH was determined as the area under the curve. A DSC (Model 2200, TA Instruments, New Castle, DE) was used to examine thermal transitions. A 10 - 15 mg solid protein sample was placed in a pre-weighed DSC aluminum pan, hermetically sealed and scanned over 30 - 150°C in the DSC at a heating rate of 5°C/min. A sealed empty pan was used as a reference.

Amino Acid Analysis

HCl Hydrolysis

A modification of the Waters Pico-Tag® method (Bidlingmeyer et al, 1984) was used for amino acid analysis. Samples were ground to pass through a 1 mm screen. About 50 - 100 mg (0.050 - 0.100 g) was weighed, depending on the protein content of the sample. Approximately 25 ml of 6N HCl was added to each sample under the hood. Nitrogen (N_2) gas was added to each tube to displace the air. A slow stream of N_2 was allowed to flow into tube for 10 seconds or more without lid, then for another 10 seconds or more with lid almost on. The lid was fastened quickly. The tubes were autoclaved for 24hrs to hydrolyze the proteins to amino acids. After hydrolysis, the pressure was allowed to come down slowly, and the samples cooled to room temperature.

The bottom of the tubes were rinsed, and dipped into water in case of acid residue. Each tube was mixed carefully on a Vortex with the lid still on. 50-ml graduated cylinders were lined up and the contents of each tube carefully transferred to the labeled graduated cylinders. 2.5 ml of 10-mM norleucine was added to each sample in the cylinder. The volume was brought up to 40 ml with HPLC water, and then mixed. Using parafilm fastened securely on top of each cylinder, they were inverted and shaken 6-8 times.

Screw-cap tubes (16mm x 125mm) in a plastic rack (1 tube per sample) were lined up and labeled. A small funnel was set into each tube, and a 7cm #2 Whatman filter paper folded and placed into each funnel. The solution was filtered carefully (at least 1 funnel full for each sample) and the unfiltered portion poured into a waste beaker under the hood so that the cylinder can be washed and used again. The filtrates were saved for derivatization.

Derivatization

Mini-tubes (6mmx50mm) were lined up in racks and labeled using a fine tip black pen only. 25 µl each of sample hydrolyzate and norleucine as internal standard was added to each tube using a new tip for each sample. 25 µl of amino acid standard, the soy protein isolate and casein controls and norleucine was added to separate tubes. The samples were dried in the speed vacuum oven set to medium for 1 hour. After drying, 20 µl of Redry solution (Table 10) was added to each dried tube and mixed. The samples were dried again in the vacuum oven for 1/2 hour.

Table 10 Preparation of Reagents for Amino Acid Analysis

Reagent	Quantity
Redry Solution	
0.5N NaAc	200 µl
MeOH	200 µl
TEA	100 µl
Derivatization Solution	
H_20	100 µl
TEA	100 µl
MeOH	700 µl
PITC	100 µl
Stock EDTA (1000 ppm)	
EDTA	10 mg
H_20	100 ml
Eluent A [1, 2]	
NaAc	19 g
H_2O	1 L
TEA	0.5 ml
EDTA	200 µl
Eluent B [2]	
Acetonitrile	300 ml
H_2O	200 ml
EDTA	100 µl
Diluent Solution	
Eluent A	100 ml
MeOH	100 ml
10 mM Norleucine	
Norleucine	mg
H_20	up to 500 ml
AA Standard H	
1:2 (0.5 STD)	1 ml STD H : 1 ml H_20
1:4 (0.25 STD)	1 ml STD H : 3 ml H_20

[1] Titrate solution to pH 6.4 with acetic acid, filter, use 940 ml and add 60 ml acetonitrile
[2] Degas under vacuum for 60 sec

The dried samples were stored in a dessicator in the freezer. Samples were equilibrated to room temperature prior to opening the dessicator to avoid water condensation on the inside and outside of the tubes. The derivatizing solution with PITC (Table 10) was made up just prior to use, and each chemical added in the order listed, mixing after each addition.

20 µl of the derivatizing solution was added to each tube quickly and without stopping between samples except to mix. Each tube was mixed carefully but quickly on vortex ensuring that no spillage occurred. The timer was set for 10 minutes after mixing was complete. The tubes were placed in a vacuum oven and the samples dried. It is recommended that only 24 tubes at a time be derivatized to keep reaction times constant. The samples were then stored desiccated in freezer until ready to run on the HPLC. When run is ready, 200 µl of the diluent solution (Table 10) was added to each tube. This was mixed on a vortex until dissolved (ideally) ensuring that no spillage occurs.

HPLC Analysis

The PITC amino acids were analyzed by direct injection with a Waters HPLC system using the following conditions: Column: YMC, C18, 5 µm, 120A°, .46x25cm; Carrier: Helium; peak sensitivity = 95; base sensitivity = 60; minimum area = 30; dilution = 1. The volume of eluent during the run was % B: 0min=2, 12min=10, 18min=25, 40min=42, 41min=100 @ 1.5ml/min, hold to 46min, 47min=2, 60min=2, 61min=2 @ 1ml/min, equilibrate @ 61min. The sample volume was 60 µl, cycle time 59.5min and

one injection per sample was used. The column heater was maintained at 46°C throughout the run. The amino acids were detected using an ultraviolet (UV) detector at a wavelength (λ) of 254 nm, and a chromatogram showing amino acid and retention time, was generated using the software package PeakSimple II, Version 3.85 (SRI Instruments, Torrance, CA). Amino acids from the experimental samples were determined using a Sigma Chemicals amino acid standard solution (A9656).

Methanesulfonic Acid (MSA) Hydrolysis - Tryptophan Analysis

The analysis of tryptophan (Trp) in proteins and peptides is complicated by the instability of this amino acid under the normal hydrolysis conditions with 6N HCl. A modification of the Waters procedure using MSA was used to generate intact Trp. About 1 mg protein for each fraction was weighed and added to an appropriate size tube. 2 ml of 4M MSA containing 0.2% (w/v) tryptamine HCl and 10 ml H_2O were added to each tube. The reaction vial was sealed for hydrolysis using the usual procedure. The mixture was hydrolyzed at 110°C (15 psi) for 24 hrs. After hydrolysis, 2.2 ml of 4M KOH (sufficient to neutralize) was added to each sample. The contents were filtered carefully to remove any suspended material. The usual derivatization procedure was then followed. A Trp solution was made up (2.5 μmol/ml) in H_2O and derivatized.

Formic Acid Oxidation - Analysis of Cysteine

Cysteine (Cys) analysis in peptide and protein samples is also complicated by the instability of the amino acid under acid hydrolysis conditions. Unfortunately, unlike Trp, acid alternatives to HCI as well as base hydrolysis are unsatisfactory. The procedure used for Cys analysis involves conversion to more stable derivatives via oxidation to the acid stable sulfonic acid, cysteic acid (Cya).

The sample (about 1 mg protein) was weighed and added to an appropriate size tube. 19 volumes of 97% formic acid was mixed with 1 volume H_2O_2 and the mixture allowed to stand covered for 24 hours. 1 ml of this reagent was added to the sample, and allowed to stand for 30 min at 22°C. The resulting mixture was dried in a Speed-Vac for 30 min. then sealed for hydrolysis using the usual nitrogen procedure. The derivatization procedure was then followed, and the samples analyzed using a Waters HPLC.

Functional Properties

Oil and Water Absorption Capacity

Flour containing 1g protein and 10-ml distilled water or oil were mixed for 30 sec in a mixer (Vari-whirl, mixing control - "fast"). The samples were allowed to stand at RT for 30 min, then centrifuged at 5000 x g for 30 min. The volume of the supernatant was recorded and the results expressed on a dry wt basis as g of water or oil absorbed / g of flour or protein (Okezie and Bello, 1988). The density of water = 1 g/ml, and oil = 0.88 g/ml.

Protein Solubility

A modification of the AACC Method 46-23 (1984) was used to determine protein solubility. A 500-mg protein sample was weighed into a 150-ml beaker. Deionized distilled water was added with stirring to form a smooth paste, then more added until the total volume of the dispersion was 40 ml. The beaker was placed on a magnetic stirrer insulated with plastic sink matting to prevent heating during the subsequent stirring period. The mixture was stirred using a 2.5 cm smooth plastic coated stir bar at a rate that just failed to form a vortex. The pH of the dispersion was measured, then adjusted to 7.0 with 0.1N HCl or NaOH. It was stirred for 1 hr under these conditions, and checked and maintained intermittently. The mixture was transferred to a 50-ml volumetric flask, and diluted to the mark with water, then mixed by inverting and swirling.

The dispersion was centrifuged at 20,000 x g for 30 min., and the supernatant filtered through a Whatman No. 1 filter paper. The protein content of the filtrate was determined by the micro-kjeldahl method, and protein solubility in distilled water calculated as - Protein Solubility (%) = {[Supernatant protein conc. (mg/ml) x 50] / [sample wt (mg) x sample protein content (%)/100]} x 100

Foaming Capacity and Stability

A modification of the method reported by Sathe and Salunkhe (1981a) was used. A 1-g protein sample and 100 ml distilled water was whipped for 5 min at high speed in a Waring Blender at RT. The mixture was poured into a 250 ml measuring cylinder, and the total volume measured at time intervals of 0.0, 0.5, 1.0, 2.0, 3.0, 8.0, and 36 hr. The

effect of concentration using 1, 2, 3, 5, 7 and 10 % (w/v) aqueous protein solutions was evaluated. Foam capacity was calculated using the following equations:

Volume increase (%) = vol. after whip - vol. before whip / vol. before whip

Emulsion Capacity and Stability

A modification of the method reported by Sathe and Salunkhe (1981a) was used. A 2-g protein sample was blended in a Waring Blender with 100-ml distilled water for 30 sec at "HI" speed. Oil in was added in 5-ml portions (from burette) and blending continued at RT until a drop in consistency was observed. The drop in consistency, judged by a decrease in resistance to blending, was taken as the point of discontinuation of oil addition. The oil added to this pt = emulsifying capacity of the sample. The emulsion was allowed to stand in a graduated cylinder, and the volume of water separated at time intervals of 10, 20, 35, 60, 100, 120, 140, 160, 180, 200, and 780 hr recorded.

Gelation Characteristics

A modification of the method reported by Sathe and Salunkhe (1981a) was used. A 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% (w/v) protein sample was prepared in 5-ml of distilled water in testubes. The mixture was heated for 1 hr in a boiling water bath followed by rapid cooling under running cold tap water. It was cooled further for 2 hr at 4°C. The least gelation concentration (Gel P) = concentration when sample from inverted tube did not fall down or slip. A texture profile analysis (TPA) of an 18% gel for each

fraction was generated from the Kramer Shear Press, and the hardness (HARD), work done on the gel (WORK), cohesiveness (COHES), adhesiveness (ADHES), gumminess (GUM), and chewiness (CHEW) determined.

Colorimetric Analysis

The color of 15-ml samples of each of the liquid fractions was measured using a Hunter Color Meter (Model D25D2) with a yellow reference standard. Color was expressed in terms of L, a and b, where L = lightness, + a = red, - a = green, + b = yellow and - b = blue.

Nutritional Characterization

Screening for Phytohemagglutinin

Phytohemagglutinin (PHA) was determined using the method reported by Occena (1994) with anti-Phaseolus vulgaris lectin (Sigma P0526) as immunogen and Phaseolus vulgaris lectin (Sigma P8629) as reference. 0.01 g/ml slurry of the protein test sample in deionized distilled water was prepared, and 40-µl aliquots pipetted into round bottom microtiter wells. Each test sample and a purified *Phaseolus vulgaris* lectin used as a reference were reacted with 10 µl of antibody. The same quantities of protein slurry were pipetted into adjacent rows of wells, but no antibody added to distinguish sample settling from agglutination. The formation of the precipitate was determined subjectively after an

hour. The results were recorded using the following notation: + indicates the presence of a precipitate; number of + indicates the degree of precipitation (++++ strong positive, + slight positive); ND indicates that precipitation was not detected; --- indicates that the sample was not analyzed.

In-vitro Protein Digestibility

The *in-vitro* digestibility of experimental samples and reference casein were measured using the four enzyme pH-Drop method outlined by AOAC (1990) and the three enzyme pH-Stat method described by McDonough et al (1990).

pH Drop Method

Cowpea and navy bean fractions, soy protein isolate and a sodium caseinate standard (Sigma C-8654) were weighed to contain approximately 10-mg nitrogen (Table 11). Ten ml of deionized distilled water was added to each test tube containing a stirring bar. Protein suspensions were held in a water bath maintained at 37°C for at least an hour.

One ml of "Enzyme A" solution (Table 12) which had previously been equilibrated to pH 8.0+0.03, was added to the protein suspension after it had also been equilibrated to pH 8.0+0.03. After incubation with Enzyme A for 10 minutes at 37°C, 1.0 ml of "Enzyme B" solution (Table 12) was added to the protein suspension. This was further incubated for 9 minutes in a water bath maintained at 55°C. At precisely 20 minutes from the addition of "Enzyme A", the pH was read at 37°C. The extent to which

pH of the protein suspension dropped was used as a measure of protein digestibility. Percent digestibility was calculated as follows:

% Protein Digestibility = 234.84 - 22.56 (X), where X = pH at 20 minute.

Table 11 Nitrogen Content and Sample Weight of a 10 mg Nitrogen sample of Cowpea and Navy Bean Fractions after Ultrafiltration Processing

Fraction	Nitrogen Content (% N)	Sample Wt (g) 10 mg Nitrogen
CPF	4.68	0.214
CPPE	6.16	0.162
CPI	9.08	0.110
CPR	3.42	0.292
NBF	4.27	0.234
NBPE	6.54	0.153
NBI	8.41	0.119
NBR	1.60	0.625
SF	6.16	0.162
SPI	14.01	0.071

Table 12 Preparation of Enzymes A and B used for pH-Drop in-*Vitro* Digestibility of Cowpea and Navy Bean Protein Fractions

Enzyme	Quantity of Enzyme (mg / 50 ml)	
	Enzyme A	Enzyme B
Porcine Pancreatic Trypsin (Type IX, Sigma T-0134	68	n/a
Bovine Pancreatic Chymotrypsin (Type II, Sigma C-4129)	166	n/a
Porcine Intestinal Peptidase (Grade K, Sigma P-7500)	26	n/a
Bacterial Protease (Pronase E)	n/a	397.5

pH-Stat Method

A solution containing all 3 enzymes (Table 13) was adjusted to pH 8.0 with 0.1N HCI and / or NaOH, maintained for exactly 2 min. then transferred to an ice bath and kept at 0°C. The multienzyme solution was prepared daily and its activity checked using an aqueous suspension of non-alkali-treated sodium caseinate (1 mg N / ml H_20). Ten ml of the sodium caseinate suspension was placed in a reaction vessel at 37°C, the pH was adjusted to 8.0 and maintained for 5 – 10 min. before adding 1 ml enzyme solution. Enzyme activity was determined from the amount of 0.1N NaOH required to maintain the pH at 7.98 for exactly 10 min. Percent true digestibility (TD) was calculated using the following equation: TD = 76.14 + 47.77B, where B = ml 0.1N NaOH added (McDonough et al, 1990).

Table 13 Preparation of Enzyme used for pH-Stat in-*Vitro* Digestibility of Cowpea and Navy Bean Protein Fractions

Enzyme	Quantity of Enzyme (mg / 50 ml)
Porcine Pancreatic Trypsin (Type IX, Sigma T-0134	68
Bovine Pancreatic Chymotrypsin (Type II, Sigma C-4129)	166
Porcine Intestinal Peptidase (Grade K, Sigma P-7500)	26

An amount of sample containing 10-mg nitrogen (Table 11) was dissolved in 2.5 ml water, then, 2.5 ml 0.2N NaOH was added and the solutions incubated at 37°C. After 30 min., 5.0 ml 0.075N HCl was added to the solutions and the pH adjusted to 8.0. Digestibility of triplicate samples of the fractions was determined by adding 1 ml of the multienzyme solution. The amount of 0.1N NaOH required to maintain the pH at 7.98 for exactly 5 min. was measured.

An uncorrected protein digestibility value (UTD) was calculated as follows: UTD = 79.28 + 40.74B, where B = ml 0.1N NaOH used during 5 min. True digestibility of the fractions was determined by multiplying the UTD value by a correction factor (f) derived from sodium caseinate, f = 100 / UTD of sodium caseinate.

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In-vivo True Protein Digestibility

Preparation of Cowpea and Navy Bean Residue

The processing scheme for production of cowpea and navy bean residue ingredients is illustrated in Figure 6. The raw cowpea and navy bean residue, which remained after alkali extraction, was cooked in kettles for 15 min at 100°C to inactivate antinutritional factors. The cooked residues were then air-dried for 48 hours at 45°C.

Preparation of diets for evaluation

The quantities used in preparation of the 10% protein (wt/wt) experimental diets are indicated in Table 14. Each diet contained 10% protein, 13.43% fiber, 10.75% fat, 3.5% mineral mix, 1% vitamin mix, 0.25% of choline bitartrate, and 0.0014% butylated hydroxytoluene. The fat source was corn oil, and arrowroot flour, the major starch source. All of the ingredients except oil were mixed for 10-min in a mixing bowl at the slowest speed to reduce dust. The oil component was added slowly and the diets mixed for an additional 25-min at slow speed. The diets were stored at 4°C in tightly sealed plastic containers prior to use. A modified American Institute of Nutrition (AIN) rodent diet AIN-93G was used as the control, and a 2% albumin diet provided an estimate of metabolic fecal nitrogen.

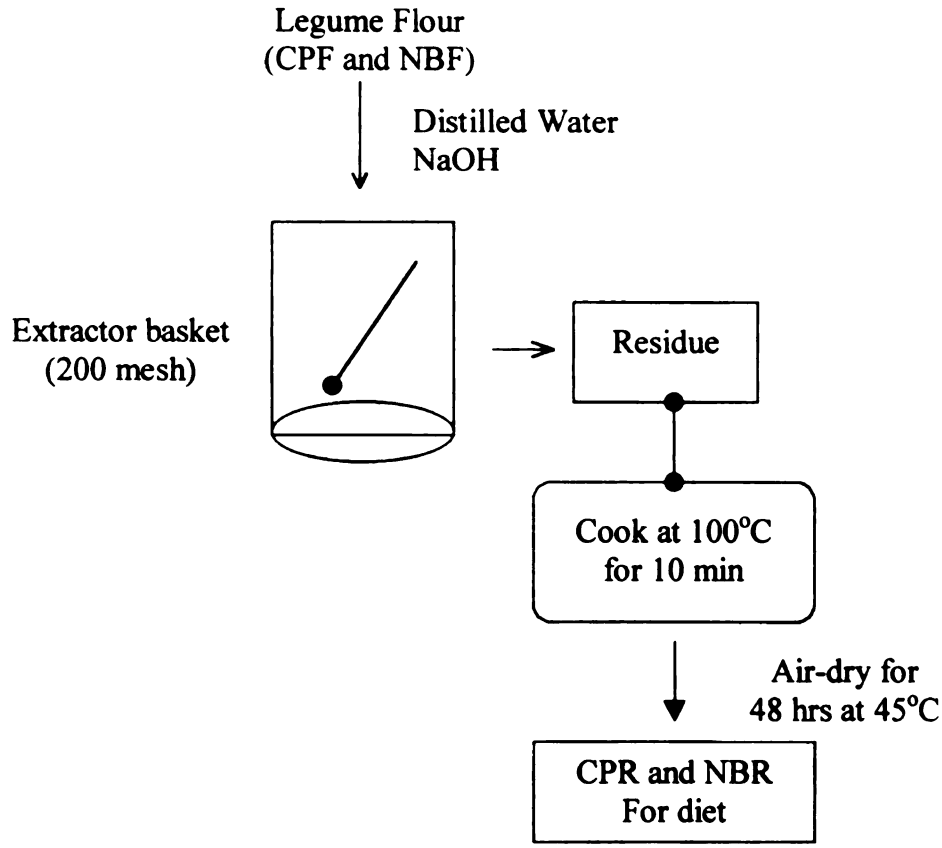

Figure 6 Processing scheme for production of cowpea and navy bean residue ingredients for *in-vivo* digestibility study

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Feeding Study

Twenty male Sprague Dawley (Harlan Sprague Dawley Inc.) rats were initially ranked by weight, 1 being the heaviest and 20 the lightest (Table 15). Each rat was randomly assigned a diet over the four-week period (Table 16), and none of them received the same diet twice. This was repeated until each diet (1 – 8) had ten rats. The protocol of the All-University Committee on Animal Use and Care (AUCAUC) on the care and use of laboratory animals was followed.

The rats were housed in individual cages and given free access to the experimental diets and water for 7 days. The jars containing diet were weighed upon the addition of fresh food. A cardboard collection box was constructed and placed under each cage to account for all eaten and spilled food, as well as fecal matter. Between days 3 and 7, a nitrogen balance study was conducted. Spilled food and fecal matter were separated, air dried, and weighed. Fecal matter collected during the nitrogen balance was pulverized to a powder with a mortar and pestle, and the protein content determined in triplicate.

Food Consumption and Rat weight

The food consumed was calculated using the initial (W_i), spilled (W_s) and final weights (W_f), i.e. food consumed = $W_i - (W_f + W_s)$. The rats were weighed on a daily basis, and the increase or decrease in weight determined over the feeding period.

Table 14

Diets are
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Table 14 Calculation of 10% Legume-Wheat Flour Diet Blends

PROTEIN CONTENT

Diets are based on 10% (w/w) protein. In 100 g of diet, the total protein will then be = 10 g.

The experimental samples have the following protein content:

WWF = 11.03 %
NBR = 15.25 %
CPR = 14.27 %

Consider protein in WWF (X):

30 % of 10 g = 3 g
X (11.03 %) = 3 g
X = 3 / 11.03 % = 27.19 g
70 % of 10 g = 7 g
X (11.03 %) = 7 g
X = 7 / 11.03 % = 63.46 g

Consider protein in NBR:

30 % = 19.67 g
70 % = 45.90 g
100 % = 65.57 g

Consider protein in CPR:

30 % = 21.02 g
70 % = 49.05 g
100 % = 70.07 g

FIBER CONTENT

The AIN-93G diet normally has 5 g of fiber, however the experimental diets will have significantly more because of the high fiber content of the samples. The fiber content of all diets will have to be based on the diet with the highest quantity of fiber. The experimental samples have the following fiber content:

WWF = 15.2%
NBR = 18%
CPR = 18%

Consider the 70:30 NBR:WWF diet
15.2 % x 27.19 g WWF = 4.13 g fiber
18 % x 45.90 g NBR = 8.26 g fiber
Total = 12.39 g fiber

Consider 70:30 CPR:WWF diet
15.2 % x 27.19 g WWF = 4.13 g fiber
18 % x 49.05 g CPR = 12.96 g fiber
Total = 12.96 g fiber

Consider the 30:70 NBR:WWF diet
15.2 % x 63.46 g WW = 9.64 fiber
18 % x 19.67 g NB = 3.54 g fiber
Total = 13.18 g fiber

Consider 30:70 CPR:WWF diet
15.2 % x 63.46 g WWF = 9.64 g fiber
18 % x 21.02 g CPR = 3.78 g fiber
Total = 13.43 g fiber

Consider 100 % NBR diet
18 % x 65.57g NBR = 11.80 g fiber
Total = 11.80 g fiber

Consider 100% CP diet
18 % x 65.57 g NB = 12.61 g fiber
Total = 12.61 g fiber

Thus all experimental diets must be based on 13.43 g fiber, which represents 8.43 g extra fiber than the 5 g AIN-93G diet. This displaces 8.43 g of digestible carbohydrate, and hence 8.43 g x 4 [1] Kcal/g = 33.72 Kcal of energy.

This is equivalent to 33.72 Kcal / 9 [2] Kcal/g = 3.74 g of fat. The fat content of the AIN-93G diet is 7%, thus in order to maintain the nutrient : energy ratio, it is necessary to increase the total fat. Thus the total fat in the diets = 3.74 g + 7 g = 10.74 g.

[1] 1g digestible carbohydrate = 4 Kcal
[2] 1 g fat = 9 Kcal

CODE:

WWW = whole wheat flour
NBR = navy bean residue
CPR = cowpea residue

Table 15 Ranking of rats according to weight[1]

Weight of rat (g)	Assigned #
112	1
110	2
110	3
107	4
104	5
100	6
100	7
99	8
98	9
98	10
97	11
97	12
97	13
97	14
97	15
96	16
96	17
95	18
95	19
94	20

[1] heaviest = 1 and lightest = 20

Table 16

Rat #
1
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20

Table 16 Rat and Diet Assignment for Feeding Study

Rat #	Diet Assignment			
	Group 1	Group 2	Group 3	Group 4
1	2%ALB	100%CP	70%NB	100%NB
2	100%NB	30%NB	70%CP	100%CP
3	100%CP	30%CP	AIN	30%NB
4	30%NB	70%NB	2%ALB	30%CP
5	30%CP	70%CP	100%NB	70%NB
6	70%NB	AIN	100%CP	70%CP
7	70%CP	2%ALB	30%NB	AIN
8	AIN	100%NB	30%CP	2%ALB
9	2%ALB	100%CP	70%NB	100%NB
10	100%NB	30%NB	70%CP	100%CP
11	100%CP	30%CP	AIN	30%NB
12	2%ALB	70%NB	2%ALB	30%CP
13	30%CP	70%CP	100%NB	70%NB
14	70%NB	AIN	100%CP	30%CP
15	70%CP	30%CP	30%NB	70%NB
16	AIN	70%NB	2%ALB	70%CP
17	2%ALB	70%CP	100%NB	AIN
18	100%NB	AIN	100%CP	70%CP
19	100%CP	2%ALB	30%NB	AIN
20	30%NB	100%NB	30%CP	2%ALB

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Calculation of Protein Quality

Apparent digestibility (AD) was calculated based on the amount of nitrogen (N) ingested and the feed intake, i.e. AD = $(N_i - F_n)/ N_I \times 100$.

True digestibility (TD) was corrected for metabolic losses in the feces, i.e. TD = $[N_i - (F_n - M_n)] / N_i \times 100$. N_i = N intake, F_n = fecal N and M_n = fecal metabolic N loss.

Relative protein efficiency ratio (RPER) and relative net protein ratio (RNPR) values were calculated using the following equations from Sarwar and Peace, (1994). RPER = (PER of test diet / PER of modified AIN-93G) x 100. The RNPR = (NPR of test diet / NPR of modified AIN-93G) x 100. PER is the weight gain of test rat / protein consumed by test rat, and NPR is the weight gain of the test rat + weight loss of 2%ALB rat / protein consumed by test rat.

PDCAAS Calculation and interpretation

Protein digestibility corrected amino acid score (PDCAAS) is calculated as the TD x amino acid score, where amino acid score is the mg of essential amino acid in 1 g of test protein / mg of the same amino acid in 1 g of reference protein. Scores for the nine essential amino acids were calculated using a human growth pattern of amino acids requirements for pre-school children (2 – 5 yr.) (FAO/WHO/UNU, 1985) as the reference proteins. Any PDCAAS above 100% is rounded down to 100% for further calculations. There is no nutritional advantage to consuming proteins with PDCAAS greater than 100% since excess amino acids are not utilized by the body, instead the nitrogen is excreted as urea, and the carbon skeleton is used for energy or stored in the body as fat.

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All proteins with a PDCAAS of 100% are high quality proteins, and are considered complete in being able to meet the essential amino acid requirements of humans.

Statistical Analysis

A Microsoft Excel 97 spreadsheet was used to compute the averages and standard deviations of all the data. The statistical analysis programs Stat View (Version 5, SAS Institute Inc., 1998) and JMP IN (SAS Institute Inc., 1996) were used to analyze the data, producing analysis of variance, factor analysis, correlations and multiple regression linear models. The post-hoc test Fisher's LSD was used for multiple comparisons of treatment means when a significant F value was obtained to determine the specific differences in extraction conditions which existed between the various treatments.

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STUDY 1 OPTIMIZATION OF THE AQUEOUS EXTRACTION OF PROTEINS FROM COWPEAS (*Vigna unguiculata*) AND NAVY BEANS (*Phaseolus vulgaris*)

Abstract

This study was conducted to evaluate the effect of pH, particle size and temperature on the efficiency of protein extraction from cowpeas (*Vigna unguiculata*) and navy bean (*Phaseolus vulgaris*) flours, and to assess optimal extraction conditions for further ultrafiltration processing. The seeds were milled to produce five different particle sizes (0.79mm - 6.35mm screens), and extracted in distilled water at six different pH values (2-12) for 60 minutes each at 25 or 50°C. Sodium hydroxide and acetic acid were used to adjust to alkaline and acidic conditions, respectively. The aqueous extracts were lyophilized and protein yield determined. Alkaline pH was more effective than acidic pH for extracting protein. Particle size had a highly significant inverse relationship to protein extraction efficiency. Temperature did not have any significant effect on protein extraction. Results indicate that the optimum protein extraction conditions for further ultrafiltration processing were particle size of 1.59 mm, at pH 10 and 25°C. Under these conditions, 87% and 91% of the total protein was recovered from cowpea and navy bean flour respectively, after three sequential extractions.

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Introduction

Protein extraction of various legume seeds has been extensively reported in the literature (Evans and Kerr, 1963; Hang et al, 1970; Fan and Sosulski, 1974; Khan et al, 1980; Sumner et al, 1981; Sauvaire et al, 1984; and Tzeng et al, 1990). Sefa-Dedeh & Stanley (1979) reported optimum conditions for protein extraction from cowpeas using various extraction solvents. Kohnhorst et al, (1990) reported on an air classification method for the production of high protein flours. However, very little work has been reported on extraction conditions for further ultrafiltration processing for these legumes.

According to Sathe et al (1984), the procedures for extracting proteins differ depending on the purpose and end use. They reported that a convenient method for food applications is the wet extraction-isoelectric precipitation method described by Fan and Sosulski (1974). Other authors have reported on dry methods such as air classification to physically separate the protein rich fraction (Sosulski and Young, 1979; and Kohnhorst et al, 1990).

Fractionation according to molecular weight for desirable functional properties was reported by Sathe et al (1984). They recommended extraction in aqueous alkaline solution followed by ultrafiltration separation of the protein. Ultrafiltration has traditionally been used in the dairy industry to recover and purify proteins from skim milk (Peri et al, 1973). It is being used increasingly with legume seeds, and is generally recommended as a low energy processing method that produces high quality protein isolates with desirable functional properties (Lawhon et al, 1979, 1981). There has been very little work reported on optimized legume protein extraction for ultrafiltration processing.

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Studies focused on optimization of protein extraction indicate that type of legume, extraction conditions (pH, time, solvent, temperature), and particle size are important parameters that affect the protein recovered (Fan & Sosulki, 1974; Sathe and Salunkhe, 1981; and Farias et al, 1995).

The null hypothesis (H_o) being tested in this study is the selected process variables (pH, particle size, bean type, and extraction temperature) do not have any effect on the yield of protein extracted from cowpea and navy bean flour.

Thus, the objective of this research was to study the effect of particle size, extraction solvent pH and extraction temperature on the efficiency of protein extraction from cowpeas and navy beans using a completely randomized experimental design.

Materials and Methods

Mature dry seeds of cowpea (*Vigna unguiculata* cv. California Blackeye No. 5) and navy beans (*Phaseolus vulgaris* cv. Huron) were purchased from Bayside Best Beans LLC, Sebewaing, MI. The seeds were milled in a standard fitzmill (0.79 – 6.35 mm screens) and the flour stored at 4°C until use.

Proteins were extracted from the flours (Figure 2), the extracts freeze dried and nitrogen content determined on a dry basis by the micro-Kjeldahl method (AACC, 1984). Protein content was calculated using the nitrogen to protein factor of 5.7, soluble solids (%) of the extracts and protein yield expressed as g protein / 100 g flour, were calculated. The optimal conditions of protein extraction were determined based on protein yield, milling time, and requirements for further processing steps.

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The experiment was repeated using three sequential extractions of the optimal conditions, and the three supernatants produced were combined. A mass balance of the combined extracts was calculated.

Statistical Analysis

In this experiment, a 6x5x2x2 factorial arrangement of treatments was used. A Microsoft Excel 97 spreadsheet was used to compute the averages and standard deviations of the data. The independent variables in the experiments included pH, extraction temperature, legume type, and particle size, while the dependent (response) factor was protein extracted. Stat View (SAS Institute Inc., 1998) and JMP IN (SAS Institute Inc., 1996) were used to analyze the data, and a multiple regression linear model fitted. If any of the independent variables were insignificant, they were removed from the analysis, and the model re-examined.

Predicted protein values were computed and leverage plots for main and interaction treatment effects generated to show the relationships between the independent variables and protein extracted. Fisher's LSD was used for multiple comparisons of treatment means when a significant F value was obtained to determine the specific differences in extraction conditions which existed between the treatments.

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Results and Discussion

The particle sizes of cowpea and navy bean flours ranged from a fine texture (0.79mm screen) to very coarse particles (6.35mm screen). Visual examination of the finest fraction showed that it had the texture of wheat flour, while the coarsest fraction had much larger particles, and tended to look and feel like split beans. Regrinding the coarser fractions through all screen sizes produced the smaller particle sizes.

Table 17 shows the proximate composition of cowpea and navy bean flours. It indicates that carbohydrate was the most significant component in both legume flours. The protein and moisture contents were higher in cowpea, while lipid and ash was higher in navy bean.

Table17 Proximate Composition of Cowpea and Navy Bean Flour (%db)

Component	Cowpea	Navy Bean
Protein	26.7 ± 0.84	24.4 ± 0.59
Lipid	2.1 ± 0.23	3.3 ± 0.35
Ash	3.5 ± 0.56	3.8 ± 0.15
Moisture	11.9 ± 1.1	8.1 ± 0.79
Carbohydrate	55.8 ± 0.68	60.4 ± 0.47

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The data on protein content of the extracts, the predicted protein from the regression model, soluble solids, and their protein recovery yield indicated that protein content ranged from 11 - 38% for cowpeas, and 11 - 41% for navy beans. The soluble solids ranged from 0.75 - 5.07% and 0.16 - 4.86% for cowpea and navy bean respectively. The protein yield ranged from 0.7 – 41% for cowpeas and 4 – 55% for navy beans. The yields of protein from cowpea and navy bean flour were generally proportional to the protein contents of the extracts. The lowest yield of 0.68 g protein / 100 g flour was reported for navy bean at pH 10, particle size 6.35 mm and 50°C. The highest yield of 55.06 g protein / 100 g flour was reported for navy bean at pH 12, particle size 0.79 mm and 25°C.

The results indicated that alkaline pH was more effective than acidic pH in extracting protein, which was due to the greater concentration of acidic amino acid residues found in legumes. Protein content increased with increasing pH except at about pH 4. The cowpea extraction curves (Figures 7 and 8) showed a well defined lowering in protein content at pH 4, however for navy bean (Figures 9 and 10), this ranged between pH 4 - 6. Although the isoelectric points were not determined, it was assumed that the pH or pH range of minimum protein content in the extract was the isoelectric point for the legume protein. The steep portion of the nitrogen extraction curves for cowpea (Figures 7 and 8) were again more pronounced than those for navy bean (Figures 9 and 10), and occurred between pH 4 - 8. These results appear to agree with the general protein extraction pattern for other legume seeds as reported by Fan & Sosulski (1974) and Sathe & Salunkhe (1981).

Figure

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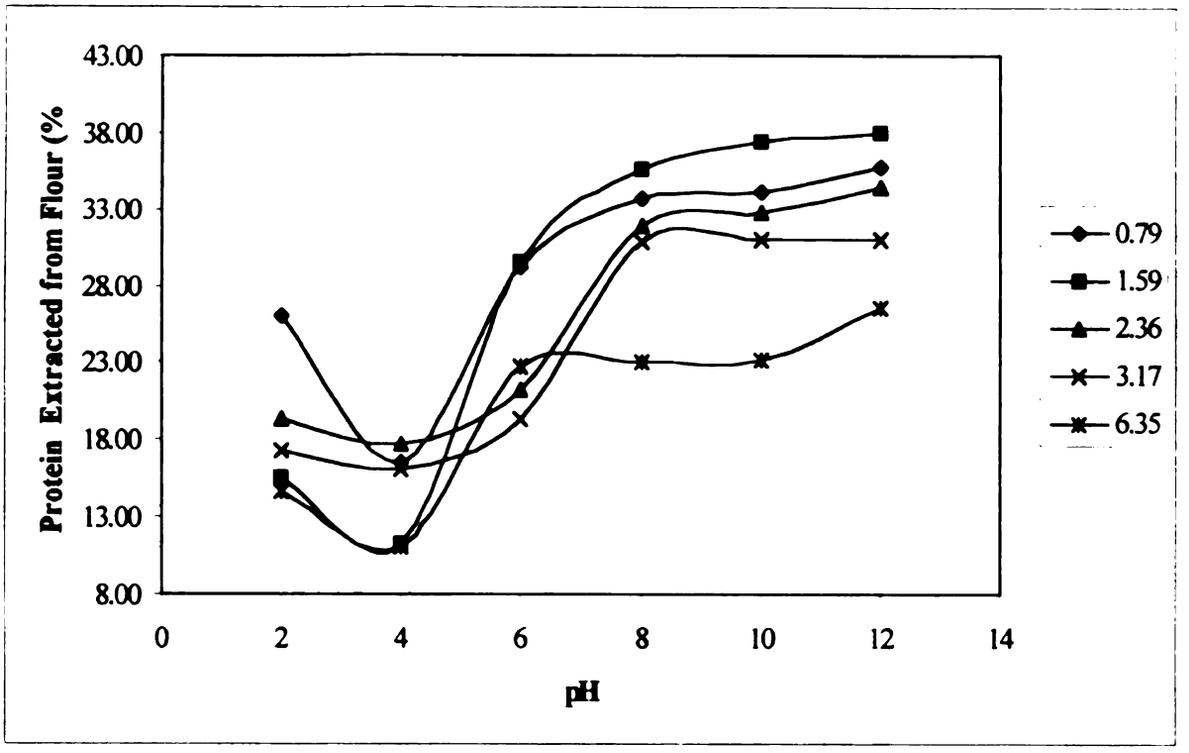

Figure 7 Cowpea Protein Extraction Curve, pH and Particle Size Effects at 25°C

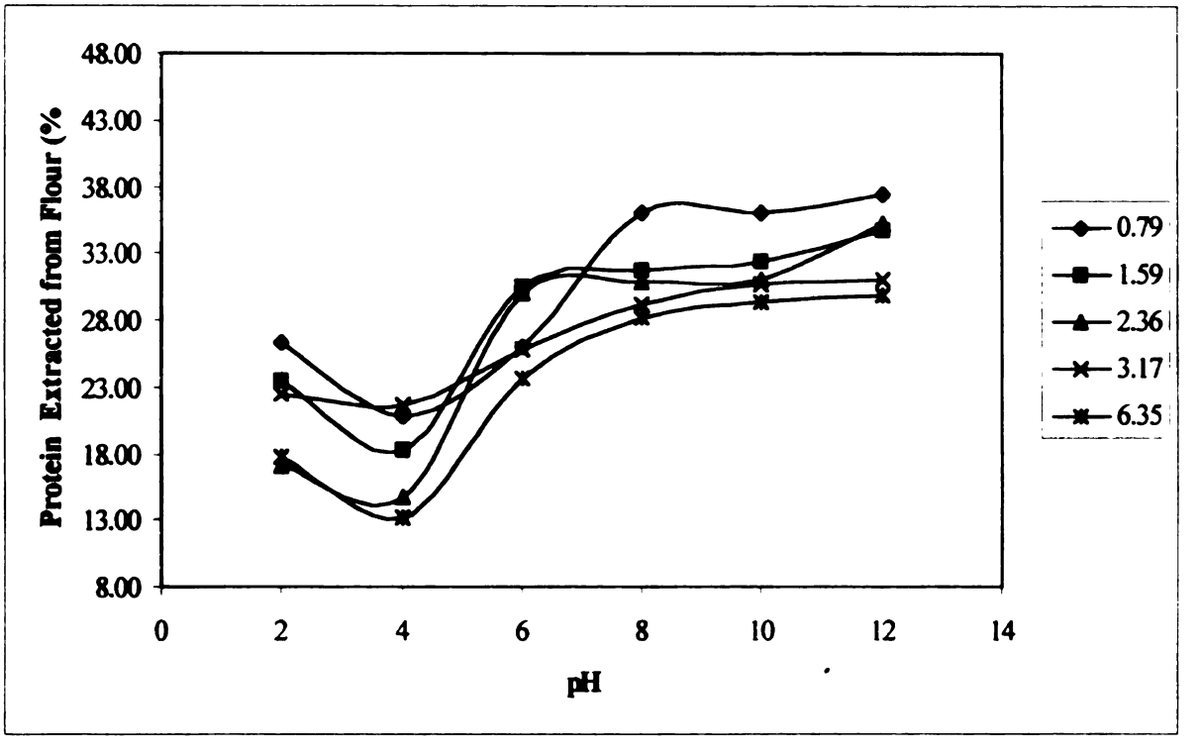

Figure 8 Cowpea Protein Extraction Curve, pH and Particle Size Effects at 50°C


Protein Extracted from Flour (%

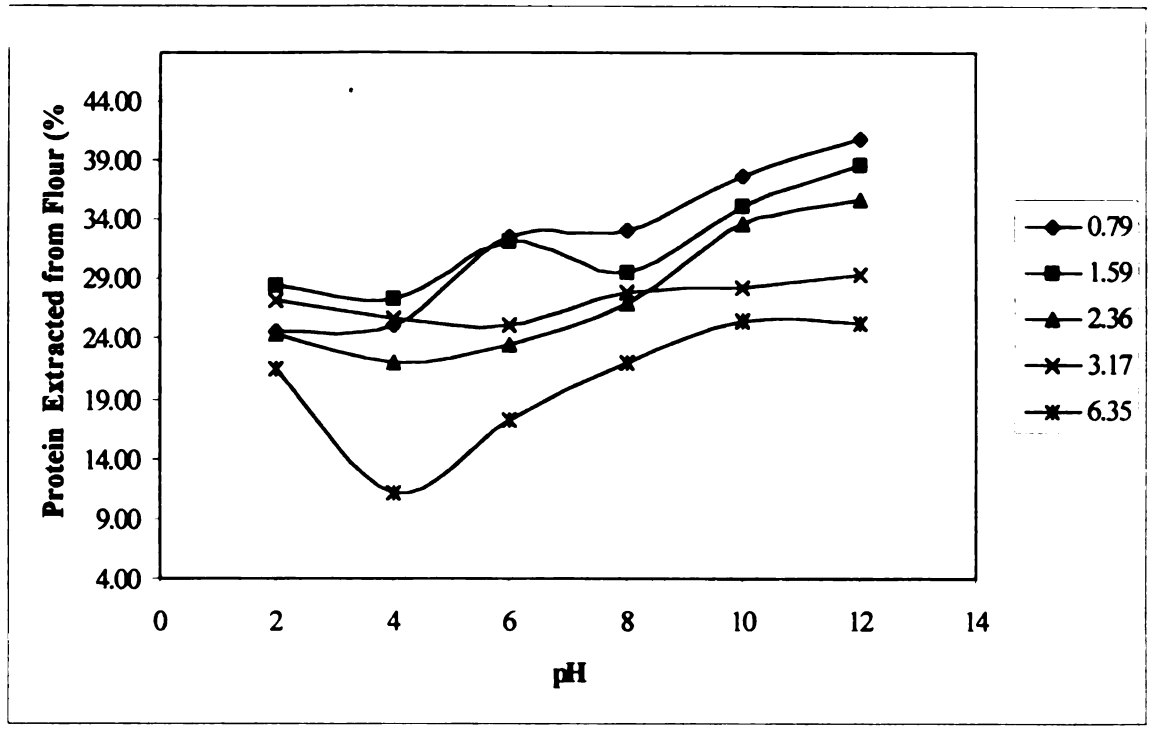

Figure 9 Navy Bean Protein Extraction Curve, pH and Particle Size Effects at 25°C

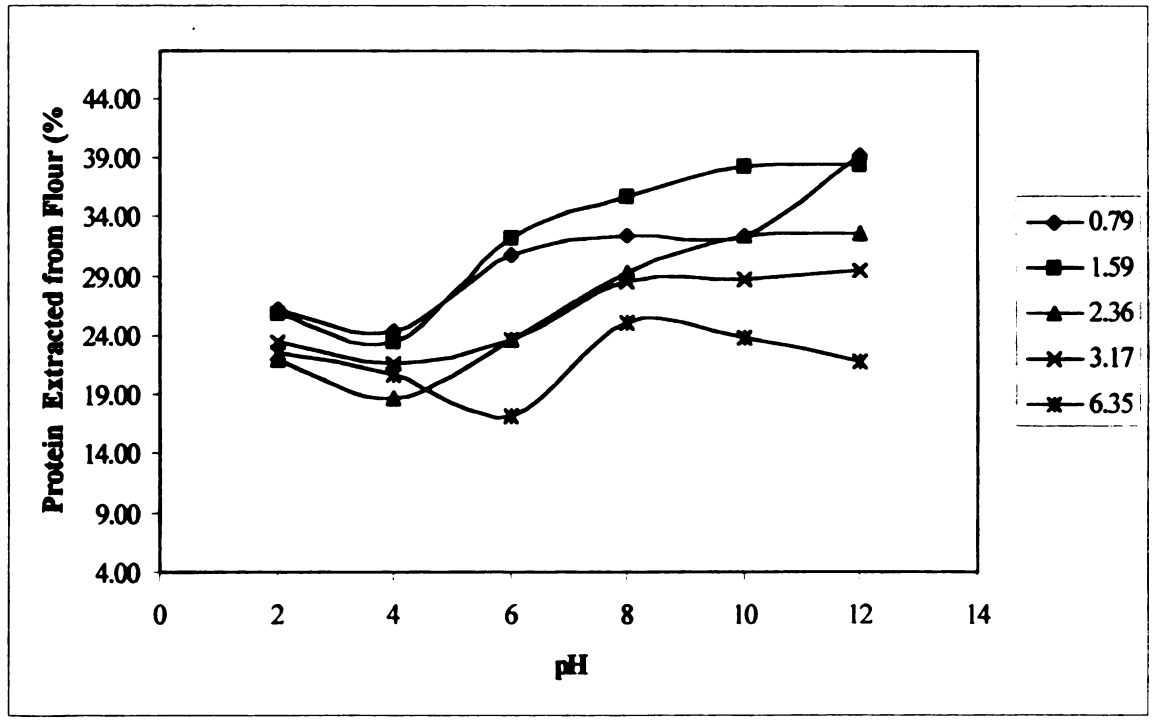

Figure 10 Navy Bean Protein Extraction Curve, pH and Particle Size Effects at 50°C

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Additionally, as particle size increased, the protein content of the extract decreased. The greater surface area available for smaller particle sizes with the extraction solvent can explain this trend. There was no definitive relationship observed for extraction temperature and protein content of the bean extracts. Overall, the higher temperature used in this experiment, did not appear to be an advantage in increasing the protein recovered.

A visual examination of the protein extracts at alkali conditions (pH 10 and 12) indicated that those for cowpea were darker in appearance than those for navy bean. This could be explained by the higher tannins content of cowpea compared to navy bean (Salunkhe, 1985).

Multiple regression was used to investigate pH, particle size, and extraction temperature, and bean type as predictors of protein content in cowpea and navy bean extract. Table 18 shows the summary of fit, and indicates that the multiple correlation coefficient (R) is 0.89, the multiple coefficient of determination (R^2) is 0.80, and the adjusted R^2 is 0.79. These are statistically significant at well under the alpha level of $p < 0.05$. $R = 0.89$ indicates that there is a good fit between the linear combination of pH, particle size, temperature, bean type and protein content. Furthermore, R^2 indicated that 80% of the variance in protein content was predictable from this linear combination. The remaining residual error is estimated to have a standard deviation of 3.14 (root mean square error).

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Table 18 Multiple Regression Summary of Fit Statistics

R	0.89
R^2	0.80
R^2 Adjusted	0.79
Root Mean Square Error	3.14
Mean of response	27.04

Table 19 shows the analysis of variance data that lists the sums of squares and degrees of freedom used to form the whole model test. The error mean squares or the variation around the regression line is 9.88 and the model mean squares is 402. The F ratio of 40.68, is highly significant ($P < 0.0001$), and suggests that the process variables overall had a significant effect on protein extraction. A leverage plot of the whole-model fit uses a scatter plot of actual response values against the predicted values to show visually the significance of the variables. It indicated that the whole-model F test was significant and also that the model fitted the data.

Table 19 Analysis of Variance of the Whole Model Test of protein content vs. the predicted protein content in cowpea and navy bean extract

Source of variation	DF	Sum of Squares	Mean Square	F Ratio
Model	11	4420.68	401.88	40.68
Error	108	1066.87	9.88	$P < 0.0001$
C Total	119	5487.55		

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R and R^2 provide valuable information on the contribution of pH, particle size, extraction temperature, and bean type taken as a group. However, it is useful to determine the independent contributions of each of the four variables. Particle size and pH had a highly significant effect ($p < 0.0001$) on protein content in the bean extracts. Bean type was significant at $p < 0.02$, but extraction temperature did not have a significant effect ($p < 0.18$) on protein extraction.

Leverage plots of the contribution of the main effects to the whole model fit indicate that the confidence curves for pH, particle size and bean type crosses the horizontal line, while that for extraction temperature does not. This further supports the conclusion that pH, particle size and bean type were significant at $p < 0.05$ level.

Table 20 shows the correlation matrix of each individual process variable, i.e. pH, particle size, extraction temperature and bean type correlated with protein content in the extracts, and each other. pH and particle size were the only process variables that showed significant correlations with protein content. pH had the highest positive correlation of 0.70 and particle size had a negative correlation of - 0.46. Both extraction temperature and bean type were poorly correlated with protein content. None of the variables showed any significant correlations with each other.

Since the previous statistical analysis reported no significant effect of temperature on protein content in the extracts, temperature was removed from the model, and the analysis recalculated. A model that included all main and interaction effects was evaluated. In this case, pH and particle size variables showed statistical significance however, there was also a significant lack of fit. The three-factor interaction effect (pH*particle size*bean) was removed from the model and then recalculated. Significant

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Table 20 Correlation Matrix of Variables in Protein Extraction of Cowpea and Navy Bean Flour

	pH	Protein	Particle Size	Temperature	Bean Type
pH	1.00				
Protein	0.70	1.00			
Particle Size	0	-0.46	1.00		
Temperature	0	0.06	0	1.00	
Bean Type	0	0.10	0	0	1.00

[1] Absolute values of correlation coefficients ≥ 0.40 were significant at 5% levels and are underlined

main and interaction effects were reported for pH, particle size, bean type, pH*bean type and pH*particle size. Lack of fit was not significant at 0.05.

Table 21 shows the protein content after three sequential extractions of 3g of cowpea and navy bean flour at pH 10 and 25°C. These data were used to calculate protein recovered from flour. Table 22 indicates that 87% of the total protein was recovered in the cowpea extract and 8% in the residue, additionally, 91% was recovered in the navy bean extract, and 5% in the residue. The remaining 5% and 4% protein unaccounted for in cowpea and navy bean respectively were presumably lost due to experimental error.

Table 2

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Table 21 Protein content in Cowpea and Navy Bean Fractions (flour particle size 1.59 mm) after three sequential extractions at pH 10 and 25°C [1]

Sample	Cowpea		Navy Bean	
	% Protein	Wt. Protein (g)	% Protein	Wt. Protein (g)
Flour	26.39 ± 0.06	0.74 ± 0.04	24.37 ± 0.09	0.68 ± 0.14
Extract 1	48.44 ± 0.31	0.34 ± 0.04	38.66 ± 0.43	0.38 ± 0.04
Extract 2	29.04 ± 0.16	0.19 ± 0.02	34.91 ± 0.06	0.15 ± 0.09
Extract 3	18.48 ± 0.02	0.12 ± 0.10	34.61 ± 0.22	0.09 ± 0.14
Residue	2.15 ± 0.30	0.06 ± 0.02	11.98 ± 0.09	0.04 ± 0.02

n = 3
[1] Initial Flour Wt = 3 g

Table 22 Protein Yield from Cowpea and Navy Bean Flour (particle size 1.59 mm) after three sequential extractions at pH 10 and 25°C

Sample	Protein Yield (g protein / 100 g flour)	
	Cowpea	Navy Bean
Extract	87.84	90.86
Residue	8.11	5.86

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Conclusions

The quantity of protein extracted from beans was significantly affected by pH, particle size and bean type. Alkaline pH had the highest protein extraction yield, while acidic pH had the lowest protein extraction yield, particularly at the apparent isoelectric point of the legume proteins. The interaction effect of pH and bean type was highly significant on percent protein extraction ($p < 0.01$), while the interaction of pH and particle size was significant at $p < 0.05$. Temperature did not have any significant effect on the protein content in the bean extracts.

The highest protein content was obtained at pH 12 and screen size 0.79 mm. However, in determining optimized conditions for protein extraction, the energy requirements involved during milling of cowpea flour, as well as heating during extraction, had to be taken into consideration. The optimal pH conditions also depended on the limits permissible for further ultrafiltration processing, and the resulting functional properties of the protein extracted.

More energy was required for production of the smallest particle size as well as heating to the higher temperature. In addition, screen size of 0.79-mm was too small for the extraction processing equipment used in this study. At pH 12, the protein would be denatured, which would affect the functional properties of the protein isolate; additionally, this is also higher than the upper limit pH for further ultrafiltration processing. The main effects of pH and particle size when investigated to determine significance of the levels, showed no significant difference between pH 10 and 12, and particle size 0.79 mm and 1.59 mm on protein content in the bean extracts. Thus, the optimum extraction conditions selected for further ultrafiltration processing was pH 10,

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particle size 1.59 mm and temperature 25°C. Under these conditions, 87% of the protein from cowpea flour and 91% from navy bean flour was extracted after three sequential extraction experiments.

H_o: the process variables, pH, extraction temperature, particle size and bean type, do not have any significant effect on protein extraction from cowpea and navy bean flours.

Reject the H_o as stated and conclude that particle size, pH and bean type had significant effects on protein extraction from cowpea and navy bean flours.

Future Research

The goals for future study include the following:

1. Conduct pilot scale optimization studies to obtain expanded data for optimal extraction conditions for further ultrafiltration processing
2. Conduct complete cost analysis to determine if the optimum protein extraction conditions are cost effective

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STUDY 2 COMPOSITIONAL AND FUNCTIONAL CHARACTERIZATION OF ULTRAFILTRATION PROCESSED COWPEA (*Vigna unguiculata*) AND NAVY BEAN (*Phaseolus vulgaris*) PROTEIN FRACTIONS

Abstract

Ultrafiltration technology is increasingly being used to simultaneously purify, concentrate and fractionate macromolecules without the application of heat or the use of either extreme chemical or physical conditions. This is extremely important in isolating proteins since it results in very little modification of structure and functionality. A plate and frame ultrafiltration system with three flat sequential membranes (molecular weight cutoff range: 5-10 KD, 15-30 KD and 50-100 KD, membrane area approximately 0.863 m^2) was used to evaluate the separation and functional characteristics of aqueous alkaline cowpea and navy bean protein extracts (CPPE and NBPE). The flow rate (L/h) and flux (LMH) of the cowpea and navy bean retentates (CPI or NBI respectively) and three permeates (CPP1, CPP2, CPP3; and NBP1, NBP2, and NBP3 respectively) were measured during a 6-hr batch process. Protein content, recovery, molecular weight characterization and functional properties of freeze-dried samples were compared to a commercial soy protein isolate (SPI). Flow rate and flux ranged from 0.19 – 2.22 L/h and 0.52 – 4.10 LMH respectively for cowpea; and from 2.45 – 9.40 L/hr and 2.83 – 10.89 LMH respectively for navy beans. Protein content of the fractions ranged from 9% - 52%. Protein recovery based on the aqueous extract, was 75.6% in CPI, and 7.49%, 3.63% and 0.67% in CPP1, CPP2 and CPP3 respectively; and 72.6% in NBI, and 4.70%, 2.14% and 1.98% in NBP1, NBP2 and NBP3 respectively. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) of the protein fractions indicated band patterns in the range

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of 6.5 - 66 KD for CPI, 14 - 20 KD for CPP1, 14.5 - 29 KD for CPP2, 14 - 55 KD for CPP3, 14 – 66KD for NBI, 7 KD for NBP1, 15KD for NBP2 and 16 –24 KD for NBP3. Water absorption capacity (WAC) and protein solubility (PS) of CPI were higher compared to SPI; however for NBI, WAC was lower and PS higher than SPI. Oil absorption capacity (OAC) for both CPI and NBI were higher than SPI. Emulsification (EC) and foaming capacity (FC) of NBI was higher than SPI. EC for CPI was higher, but FC lower than SPI. The critical protein concentration for gelation and gel strengths of CPI and NBI were lower than SPI. Thermal stability was greatest for CPI, then NBI and SPI. Amino acid profiles for the fractions were similar and increased after extraction and UF processing. The plate and frame ultrafiltration membrane system employed, provided discrete protein fractions with functional properties.

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Introduction

Ultrafiltration technology (UF) is used to simultaneously purify, concentrate and fractionate macromolecules without the application of heat or the use of either extreme chemical or physical conditions. This is particularly important in isolating proteins since it results in very little modification of structure and functionality. It is being increasingly utilized in the food industry to separate molecules through a porous polymeric membrane on the basis of their molecular weight. It results in concentration of higher molecular weight solutes in the concentrate, and the production of a lower molecular weight permeate stream, depending on the molecular weight cutoff (MWCO) of the membrane (NFPA, 1993).

UF is now a well accepted processing operation in the dairy industry. It is used in the fractionation of cheese whey and pre-concentration of milk for cheese manufacture, and is well reported in the literature (Timmer, 1997; Simbuerger et al, 1997; Cheryan & Chiang, 1984; Ernstrom, 1985, 1986). In the fruit industry, Yu et al, (1997) and Gao et al, (1997) reported on the use of UF for West Indian cherry juice and apple juice processing, respectively. In vegetable protein processing, UF has been increasingly utilized as a replacement for conventional isolation methods. Lawhon et al (1981, 1982) reported on the use of a combined aqueous extraction procedure to produce protein and oil food products from un-defatted soybeans. They also used UF membranes to co-process soy protein extracts and skim milk to produce a soymilk food ingredient. Lui et al (1989), and Kwon et al (1996), reported on the UF conditions for processing soy and coconut protein isolates, respectively, to improve protein functionality.

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More recently, research has been expended on leguminous species other than soybeans. Bolles (1997), reported on the quality of a navy bean retentate, and found that it was of similar quality and functionality as a soy protein isolate and could be utilized successfully in an aseptic processed infant food drink. Ko et al, (1994) reported on the use of UF to recover protein from simulated waste water during mung bean starch preparation. They found that protein recovery improved significantly, utilization of mung bean increased, and pollution problems reduced. Juarez and Lopez (1994) and Berot et al (1987) showed that the stability of juice from the tropical legume jicama improved, and yields of fababean protein isolates were higher with improved functional properties after UF processing, respectively.

In this study, the separation performance of flat plate membranes used in UF processing of cowpea (*Vigna unguiculata*) and navy bean (*Phaseolus vulgaris*) protein extracts was evaluated. The physico-chemical properties were then determined to assess the native status of the protein isolates, and their suitability as food protein ingredients.

The null hypothesis (H_o) being tested is there is no difference in the physico-chemical properties of ultrafiltered cowpea and navy bean protein isolates compared to a soy protein isolate.

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Materials and Methods

Cowpea and navy bean flour milled in a Fitzpatrick mill (Model D Comminuting Machine, Fitzpatrick Co., Chicago, IL) with screen size 1.59 mm was prepared and stored at 4°C prior to use.

The bean flours were extracted three times in extractor baskets in an aqueous alkali solvent mixture, combined then sedimented overnight at 4°C. The combined extracts were pumped through three series of flat plate membranes (Osmonics Inc., California) with molecular weight cut off (MWCO) points of 5-10KD, 15-30KD and 50-100KD respectively, and total surface area 0.863m^2. The flow rates were measured and flux calculated. The UF unit was cleaned after each run using the acid/alkali cleaning schedule recommended for protein products (EcoLab, 1992).

The fractions were freeze-dried, protein content of the samples were determined using the micro-kjeldahl method (AACC, 1984), and a protein mass balance calculated from this. SDS-PAGE was used to determine the molecular weight range of the fractions, and thermal properties were determined using a DSC Thermal Analyzer Model 2200 (TA Instruments, New Castle, DE).

Functional properties including water and oil absorption capacity, protein solubility, foaming capacity and stability, emulsion capacity and stability, gelation and colorimetric characteristics were determined for all fractions (Sathe & Salunkhe, 1981; Sathe et al, 1982; Coffman & Garcia, 1977; Beuchat, 1977; AACC, 1983).

The amino acid profile of each fraction was determined using acid hydrolysis, methanesulfonic acid (MSA) hydrolysis for tryptophan and formic acid oxidation prior to acid hydrolysis for cysteine. Phytohemagglutinin screening was measured using the

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method reported by Occena (1994) with Sigma Chemical kits P0526 and P8629. *In-vitro* digestibility was determined by the four enzyme pH-Drop method (AOAC, 1990) and the three enzyme pH-Stat method described by McDonough et al (1990).

Statistical Analysis

Microsoft Excel was used to compute the averages and standard deviations of the UF processing data. Stat View (SAS Institute Inc., 1998) was used to analyze the protein content data, functional and nutritional properties and determine differences between samples using Fisher's LSD. Factor analysis from Stat View (SAS Institute Inc., 1998) was used to determine which of the functional properties were the principal components most affected by ultrafiltration processing. Correlation matrices of the functional, nutritional and thermal properties of the fractions were generated.

Results

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Results & Discussion

The flux data for cowpea and navy bean is illustrated graphically in Figures 11 and 12 respectively. In both cases, flux decreased particularly during the initial part of the experiment. Within about 1/2 hr the flux was significantly reduced for all three permeates, then remained almost constant during final concentration. This was also reported for ultrafiltration of fababean protein extracts by Berot et al (1987) using a mineral membrane and for peanut protein products by Lawhon et al (1981).

This rapid decrease in flux suggests possible plugging of the membrane as the CPI and NBI concentrations increased, and occurred as soluble protein in the aqueous extract formed a gel during UF processing which lead to fouling of the membrane. Berot et al, (1987) described this phenomenon in terms of the structure of this gel or polarization layer and suggested that it was dependent on the conformation of the proteins.

The lower molecular weight components all had higher flux rates. Similarly, the mean flow rates and flux for NBP3 and NBI were significantly higher than the corresponding values for CPP3 and CPI. This can be explained by the molecular weight differences between NB and CP. The molecular weight distribution from SDS-PAGE indicates that components in the navy bean fractions have lower molecular weight distribution than cowpeas. This supported the study by Lawhon et al, (1981). They found that during membrane processing of soybean proteins, the mean flux was higher with lower molecular weight extracts. Kwon et al (1996) also reported on higher initial flux rates using a 10KD membrane compared to a 5KD, during hollow-fiber UF processing of

Flux (L./m^2/hr)

Figure

Flux (L/m^2/hr)

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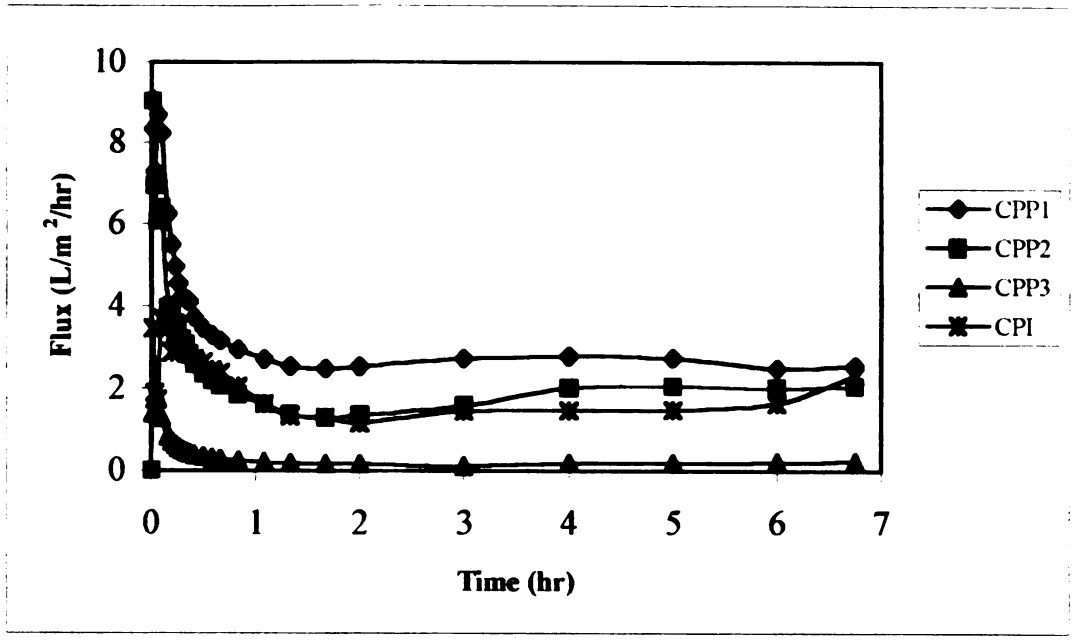

Figure 11 Flux of Cowpea Protein Fractions during Ultrafiltration Processing

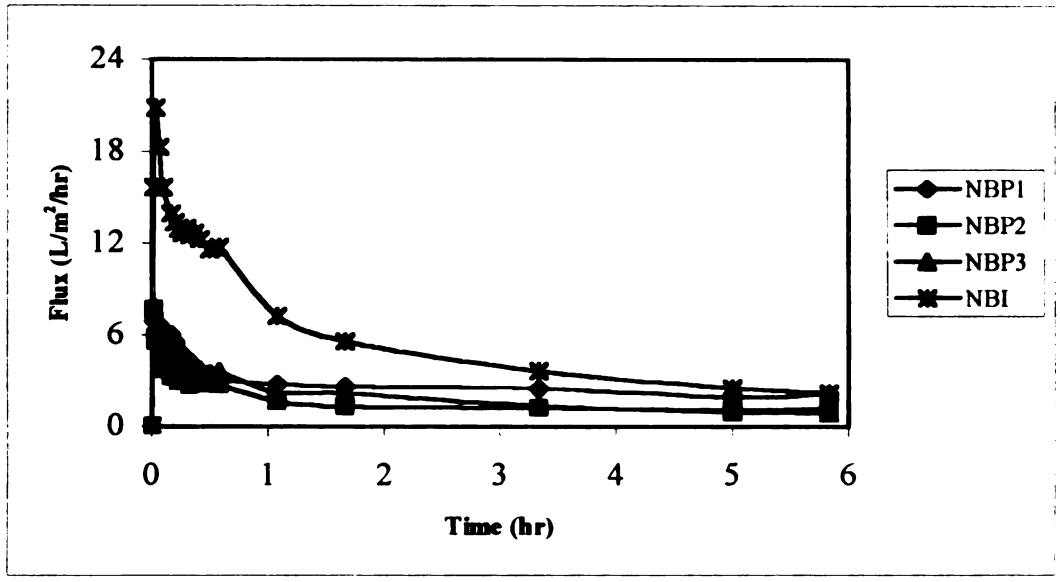

Figure 12 Flux of Navy Bean Protein Fractions during Ultrafiltration Processing

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coconut proteins. As UF operation time increased, the 5KD membrane yielded higher and more stable flux rates.

Cowpea protein extract (CPPE) was concentrated from 45L to 14L of retentate (CPI) after a 7-hr batch process. The volume of CPPE separated into permeates 1, 2, and 3 (CPP1, CPP2, CPP3) was 15L, 12L and 1.32L, respectively. The mean flow rate (L/hr) for CPP1, CPP2, CPP3 and CPI were 2.22, 1.78, 0.19, 2.03 L/hr. The navy bean protein extract (NBPE) concentrated from 32L to 11L of retentate (NPI) after a 6-hr batch process. The volume of NBPE separated into permeates 1, 2, and 3 (NPP1, NPP2, NPP3) was 10.5L, 5.7L and 4.3L, respectively. The mean flow rates (L/hr) for NPP1, NPP2, NPP3 and NPI were 3.54, 2.45, 2.94 and 9.40 L/hr respectively. The mean flux rates for cowpea were 4.10 LMH, 3.03 LMH, 0.52 LMH and 2.25 LMH for CPP1, CPP2, CPP3 and CPI respectively. The corresponding values for navy bean were 4.07 LMH, 2.83 LMH, 3.41 LMH and 10.89 LMH for NPP1, NPP2, NPP3 and NPI respectively.

Table 23 shows the protein content (dry basis) and % solids of cowpea and navy bean protein fractions after pilot plant protein extraction at pH 10 and 25°C and ultrafiltration processing respectively. The protein content of CP fractions was greater than NB. CPI had a protein content of 52% and NB had 48%, compared to 89% for the commercial soy protein isolate. The values for the experimental samples were lower than what was reported for other legume products. Berot et al (1987) reported protein contents of 85% - 91% for fababean protein isolates, Lawhon et al (1979) 80% - 92% for soy isolate from toasted and untoasted soy flour and Vose (1980) 89% and 94% for field pea and horsebean isolate respectively.

Table 23 ***Protein Content and % Solids of Cowpea and Navy Bean Protein Fractions after Aqueous Extraction and Ultrafiltration Processing***

Components

Table 23 **Protein Content and % Solids of Cowpea and Navy Bean Protein Fractions after Aqueous Extraction and Ultrafiltration Processing**

Components	Cowpea		Navy Bean	
	% Protein	% Solids	% Protein	% Solids
Flour	26.67 ± 0.03	88.52 ± 0.89	24.37 ± 0.03	89.15 ± 0.99
Extract 1	41.87 ± 1.61	2.86 ± 0.47	33.33 ± 1.61	4.82 ± 0.47
Extract 2	35.58 ± 2.78	1.16 ± 0.50	30.10 ± 2.78	2.29 ± 0.44
Extract 3	25.61 ± 5.85	0.48 ± 0.13	29.83 ± 5.85	1.01 ± 0.05
Combined Extracts	35.14 ± 10.33	2.20 ± 1.09	37.29 ± 10.33	1.93 ± 1.09
Residue	19.49 ± 1.24	22.01 ± 1.96	9.13 ± 1.24	19.01 ± 1.96
Permeate 1	16.52 ± 2.73	0.50 ± 0.26	18.45 ± 1.03	0.65 ± 0.26
Permeate 2	13.65 ± 3.50	0.53 ± 0.23	11.18 ± 2.23	0.63 ± 0.23
Permeate 3	19.37 ± 3.88	0.50 ± 0.12	22.12 ± 1.58	0.40 ± 0.12
Retentate	51.76 ± 0.35	1.81 ± 1.27	47.96 ± 0.45	3.16 ± 0.32

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An analysis of variance (Table 24) indicates that ultrafiltration processing had a highly significant effect ($p < 0.0001$) on the protein content of the fractions. Fishers LSD indicated that all of the samples were significantly different from each other except CPP1 and CPR.

Table 24 Analysis of Variance of Cowpea and Navy Bean Fractions after UF Processing

Variance	DF	SS	MS	F Value	P Value
Fraction	14	17603.76	1257.41	14801.19	< 0.0001
Residual	30	2.54	0.08		

Figures 13 and 14 illustrate the protein mass balance of cowpea and navy bean protein fractions after UF processing. It indicated that about 63% and 52% of the total protein in cowpea and navy bean flour respectively was recovered in the extract, and 33% and 41% in the residue respectively. Recoveries based on the total protein in the flour were 47.2%, 4.7%, 2.3% and 0.4% for CPI, CPP1, CPP2 and CPP3 respectively; while 37.9%, 2.4%, 1.1% and 1.0% were received for NBI, NBP1, NBP2 and NBP3 respectively. The total loss of protein during extraction and UF processing was 13% and 17% for cowpea and navy bean respectively.

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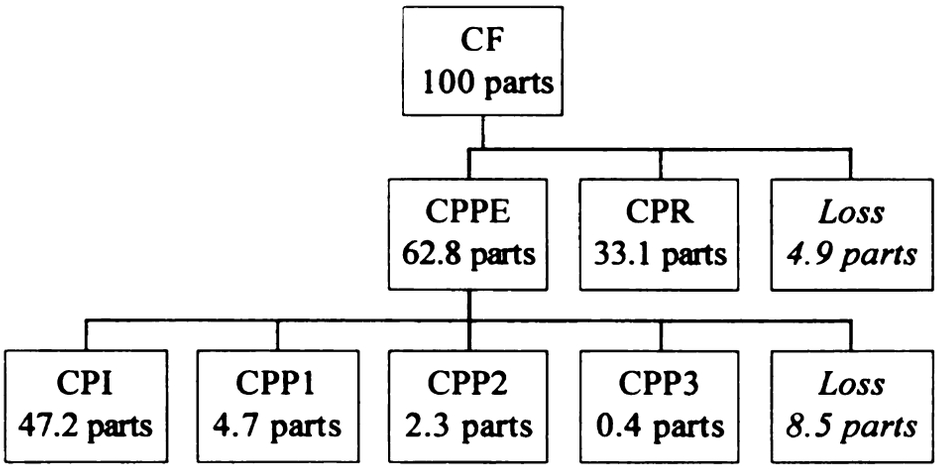

Figure 13 Mass Balance of Cowpea Protein Fractions after Ultrafiltration Processing

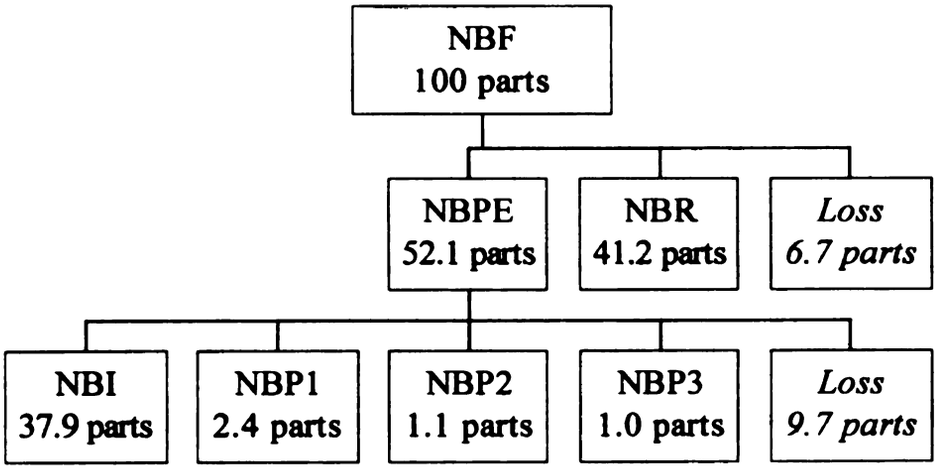

Figure 14 Mass Balance of Navy Bean Protein Fractions after Ultrafiltration Processing

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The total protein recovered from CPPE and NBPE was 87% and 81% respectively. About 75% of the total protein was recovered in CPI, and 7.5%, 3.6% and 0.7% recovered in CPP1, CPP2, CPP3 respectively. This was similar for the navy bean fractions, about 73% was recovered in NBI, and 4.7%, 2.1%, and 2% recovered in NBP1, NBP2, NBP3 respectively. These values agreed with experiments reported for chickpeas (Romero-Baranzini et al, 1995).

Figure 15 shows a photograph of the SDS-PAGE pattern from cowpea and navy bean protein fractions in the presence of 2-ME (2-mercaptoethanol). The pattern for cowpea flour, extract, residue and isolate showed distinct bands at about 97KD, a major triplet band at 62KD, 56KD, 52KD, and a doublet band at 36KD and 32KD which corresponds mainly to the globulin and albumin fractions. Nine other protein bands were observed in the cowpea flour and ranged from 156KD – 38KD. However these were much less prominent than the subunits previously described. Two of these bands in the flour fraction were greater than the 205KD protein standard. The extract, residue and isolate had four other bands that were similar to the flour fraction and corresponded to 106KD – 38KD.

The SDS PAGE pattern for the navy bean fractions showed distinct doublet bands at 98KD and 96KD and again 50KD and 46KD. A single band was observed at 33KD. Other bands observed ranged from 156KD – 39KD. The residue fraction produced bands only at 50KD, 46KD and 39KD. A similar molecular weight profile was reported for navy bean meals, extracts and concentrates by Kornhorst et al (1990) after air classification and isoelectric precipitation of navy bean meal.

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Figure 16 shows the pattern for the cowpea and navy bean permeates. No protein bands were observed on the gel for permeates 1 and 2 for both legumes (CPP1, CPP2, NBP1 and NBP2). CPP3 showed distinct bands at 62KD, 56KD, 52KD and 36KD that were similar to bands in CPF; and NBP3 showed bands at 50KD and 33KD that were similar to the major bands in NBF.

The MWCO points of the three series of flat plate membranes used included 5KD – 10KD, 15KD - 30KD and 50KD - 100KD respectively. The SDS-PAGE profile reported above, reflects the MWCO ranges with some changes in selectivity due to the concentration polarized layer as reported by Blatt et al (1970), as well differences in actual molecular weight during manufacture of the membrane. This suggests that the UF membranes were able to fractionate the protein extracts according to their molecular size. All components smaller than 10KD, 30KD and 100KD should pass through the membranes and produce permeates 1, 2 and 3. However, the lowest molecular weight observed on the gel was about 36KD which explains why no bands were observed for permeates 1 and 2. The isolates for both legumes showed a wide molecular weight profile that was similar to the corresponding flour fractions.

These data also indicate that there is a difference in the protein molecular weight profile of navy bean and cowpea proteins. This was expected since the legumes are two distinct species. The molecular weight of the major subunits in navy beans was generally lower compared to the major bands in the cowpea fractions. Kohnhorst et al (1990) and Khan et al (1980) also reported these molecular weight ranges for navy beans and cowpeas respectively.

Figure 15 SDS-PAGE Pattern of Cowpea and Navy Bean Protein Fractions after Ultrafiltration Processing (Sigma M-4038 Standard (1), cowpea flour (2), cowpea protein extract (3), cowpea residue (4), cowpea protein isolate (5), navy bean flour (6), navy bean protein extract (7), navy bean residue (8), navy bean protein isolate (9)

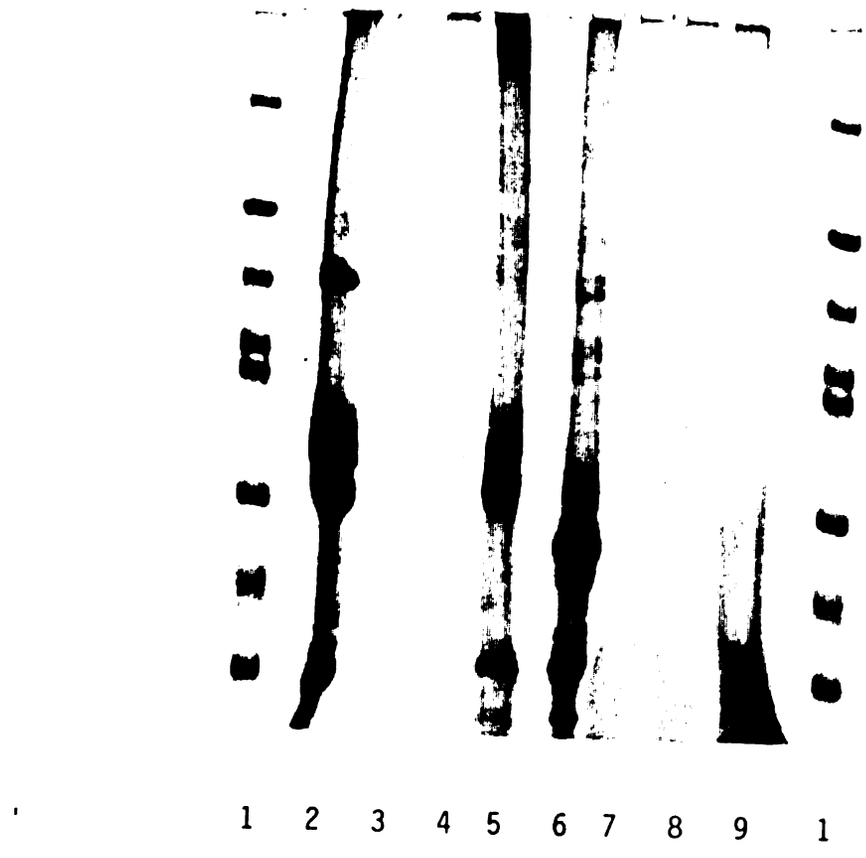

Figure 16 SDS-PAGE Pattern of Cowpea and Navy Bean Permeate Fractions after Ultrafiltration Processing (Sigma M-4038 Standard (1), cowpea flour (2), cowpea permeate 1 (3), cowpea permeate 2 (4), cowpea permeate 3 (5), navy bean flour (6), navy bean permeate 1 (7), navy bean permeate 2 (8), navy bean permeate 3 (9)

The thermograms of 10-mg solid samples of cowpea, navy bean and soybean protein fractions were similar to the generalized thermal curve for legume globulin reported by Petruccelli and Anon (1996). The peaks for all samples did not demonstrate the characteristic broadening expected with denaturation, these data suggests that the samples were not completely denatured. Nagano et al (1994) and Wang and Damodaran (1991) reported that broadening peaks indicated a shift in conformation of the protein to the unfolded state, which is associated with hydrogen bond disruption in protein denaturation.

The thermal properties of the fractions are presented in Table 25. They indicate that initial transition temperatures (T_i) ranged from 82.9°C – 105°C, peak transition temperatures (T_p) ranged from 93.5°C – 125°C, and enthalpies ranged from 0.33 – 0.55 W/g. The transition temperatures were higher and the ΔH values lower than what has been reported for other legume proteins. This was due to the moisture content of the samples in this study, which ranged from 3-5%. This was significantly lower compared to samples in other studies in which slurries of up to 20% moisture content were used (Gorinstein et al, 1996; Martinez and Anon, 1996; Liao et al, 1996; Erdogdu et al, 1995; and Okechukwu and Rao, 1997).

It has been reported that lowering moisture content increases the thermal stability of proteins (Arntfield et al, 1985) resulting in higher transition temperatures. They reported that as water is removed from a protein system, there is insufficient water in the vicinity of the protein molecule to bring about the thermal transition. As protein denaturation occurs and the protein unfolds, there is a transfer of hydrogen bonds from protein-protein to protein-water. If there is limited water in the system, then this reaction

is only possible to a limited extent resulting in lower ΔH values. Further, at low moisture levels, water is held tightly due to its association with the protein. This suggests that the energy that is required for mobilizing water is increased, resulting in elevated transition temperatures.

Table 25 Transition Temperatures and Enthalpies of Cowpea and Navy Bean Fractions after Ultrafiltration Processing [1]

Samples	T_i (°C)	T_p (°C)	ΔH (W/g)
CPPE	82.9 ± 1.27 a	94.15 ± 1.34 a	0.37 ± 0.01 a
CPI	97.0 ± 2.82 b	111.5 ± 3.53 b	0.34 ± 0.03 a,b
NBPE	91.5 ± 1.20 b,c	115.7 ± 0.99 b,c	0.33 ± 0.01 c
NBI	104.5 ± 0.71 d	125 ± 2.83 d	0.52 ± 0.02 d
SPI	105 ± 4.24 d,e	116 ± 2.83 b,c,e	0.55 ± 0.03 d,e

[1] n = 2
Means within the same column followed by different letters are significantly different ($p < 0.05$)

UF processing had a significant effect on the thermal stability of the fractions ($p < 0.05$). Both transition temperatures and ΔH values increased for all samples after extraction and UF processing. Navy bean fractions had higher transition temperatures and enthalpies overall than the cowpea and soy fractions, which indicates that these samples unfolded more during extraction and UF processing. There were no significant differences in T_i between NBI and SPI and NBPE and CPI. For T_p, there were no significant differences between NBPE and SPI; and CPI, SPI and NBPE. ΔH for CPI and

CPPE was significantly different from both NBI and SPI; however NBI and SPI were not significantly different from each other. Additionally, ΔH for CPPE and CPI were not significantly different. These results indicated that denaturation after processing was more significant with navy bean and soybean fractions than cowpea fractions.

Arntfield and Murray (1981) observed an increase in transition temperatures after heat processing. They suggested that the increase observed was due to the removal of low temperature denaturing proteins during the heat process, which resulted in higher average transition temperatures for the remaining proteins. In this present study, although there was no heating, low temperature proteins may have separated into permeates after extraction and UF processing resulting in higher temperature proteins in the isolates.

The results on ΔH support the study by Murray et al (1985) on thermal behavior of fababean and field pea proteins during purification. They reported an increase in the ΔH values as protein content increased during the purification process, and found a significant correlation between protein content and ΔH. In this study, a significant correlation of 0.77 ($p < 0.05$) was found between ΔH and protein content of the fractions. This could be interpreted as an increase in the proportion of denatured protein as protein content increases, which is reflected by an increase in ΔH as measured by DSC.

The amino acid chromatograms of cowpea and navy bean fractions were well resolved and allowed identification and good quantification of the amino acids present in the legume fractions. Due to instability to acid hydrolysis, cysteine and tryptophan were either absent or present at very low levels in all fractions, and in addition, proline was absent in both NBPE and NBI, while histidine was absent in NBI. The amino acid

composition for standard hydrolyzate, cowpea fractions, navy bean fractions, soy isolate and casein, expressed as mg amino acid / 100g protein is presented in Table 26. The amino acid profile of casein, soy isolate and the flour fractions corresponded to what has been reported in the literature (Mnenbuka and Eggum, 1995; Patel et al, 1980; and Elias et al, 1976).

The amino acid profile of CPF, CPPE and CPI reflected similar types of amino acids, which increased after extraction and ultrafiltration processing. This was not observed overall for navy bean fractions. The profile of NBPE and NBI did not reflect similar amino acids compared to NBF. Proline was not detectable in either NBPE or NBI. Alanine and methionine was either not detected or reported in very low levels for all fractions. Changes in retention and resolution for navy bean may have been due to poor resolution of alanine with proline, or by sample interference from components such as lipids, minerals and other non-proteinaceous materials. Both cowpea and navy bean fractions were richest in aspartic acid, glutamic acid, lysine and valine, and contained lowest levels in the sulfur amino acids and tryptophan.

These results suggest that UF processing concentrated the quantity of each amino acid significantly from flour to isolate. However, the amino acid content of these fractions was still considerably lower compared to those for SPI and casein. The amino acid profile observed for the flour fractions was similar to what has been reported in the literature for cowpea and navy bean flours (Maneepun et al, 1974; Hang et al, 1980; Kanamori et al, 1982).

The data for protein solubility (PS) is shown in Table 27. PS indicates how the protein will perform when applied in particular to liquid food systems and is also a

Table 26 Amino Acid Composition (g / 100g protein) of Cowpea and Navy Bean fractions

Amino Acid	CPF	CPPE	CPI	NBF	NBPE	NBI	CPR	NBR	SPI	CASEIN
Asp	11.19	16.52	15.89	15.25	15.98	8.12	18.89	11.81	11.88	10.26
Glu	15.25	21.67	21.34	16.96	23.25	8.94	21.43	14.24	15.28	11.06
Ser	5.01	5.48	5.50	7.25	5.69	1.11	6.17	5.46	2.97	6.02
Gly	5.13	5.83	5.41	5.87	4.10	0.97	6.12	4.58	2.82	3.04
His	6.53	7.61	6.96	6.30	1.60	1.35	7.53	4.52	3.85	3.25
Arg	5.05	5.88	5.78	7.31	11.65	4.80	6.05	5.07	2.27	5.83
Thr	5.52	6.26	6.10	6.95	6.47	2.93	6.81	5.23	2.60	4.06
Ala	1.01	ND	ND	ND	2.90	1.28	0.44	0.27	ND	0.33
Pro	3.76	3.85	3.81	3.82	ND	ND	3.97	2.77	2.74	9.04
Tyr	2.49	2.95	3.24	2.99	1.23	1.58	3.24	2.77	2.29	5.55
Val	4.72	6.56	6.62	6.84	6.15	2.62	6.54	5.02	3.49	6.72
Met	1.20	ND	ND	ND	1.61	ND	1.35	0.81	ND	2.66
Ile	4.39	5.69	5.67	6.01	4.61	2.06	5.74	5.28	2.27	3.20
Leu	7.18	8.23	8.37	9.51	7.74	3.44	9.06	8.55	3.66	1.57
Phe	6.49	7.80	7.79	8.15	6.93	3.01	8.23	5.97	14.09	6.41
Lys	6.42	7.61	7.62	8.02	6.30	2.73	8.19	6.06	4.21	8.32

practical measure of the extent of protein denaturation due to processing. The higher solubility of the experimental fractions compared to SPI at neutral pH indicates that CPI and NBI were less denatured than SPI, and would be useful in liquid food formulations. This data agreed with the results from the thermal study, which indicated that NBI and SPI were more denatured than CPI. These findings were also reported by Kohnhorst et al (1990) after air classification of navy and kidney bean flours.

Table 27 Protein Solubility of Cowpea and Navy Bean Protein Fractions at pH 7 [1]

Fractions	Protein Solubility (%)
CPF	39.39 ± 0.37 a
CPPE	25.04 ± 0.53b
CPI	52.09 ± 0.88 c
NBF	22.44 ± 0.35 d
NBPE	16.25 ± 0.40 e
NBI	36.26 ± 0.63 f
SPI	16.61 ± 0.21 g, e

[1] n = 3
Means followed by different letters are significantly different ($p < 0.05$)

Tables 28 and 29 present the data for water and oil absorption capacity (WAC and OAC) respectively, and indicates that WAC of the cowpea isolate (CPI) was significantly different from the soy isolate (SPI) and the navy bean isolate (NBI). This is related to protein denaturation of the SPI and NBI fractions as a result of isoelectric precipitation production for SPI and increased shearing during UF processing for NBI. The OAC of CPI and NBI were significantly higher than SPI. This agreed with other work reported by

Berot et al, (1987) and Sathe et al, (1982) for Fababean and the Great Northern Bean protein isolates respectively. These values are good indicators that the test proteins could be incorporated into food formulations like doughs and meat extenders.

Table 28 Water Absorption Capacity of Cowpea and Navy Bean Protein Fractions [1]

Fractions	WAC ($g H_2O$ / g protein)		
	Cowpea	Navy Bean	Soybean
Flour	3.75 ± 0.01 a	8.21 ± 0.66 d	---
Extract	2.36 ± 0.25 b	6.70 ± 0.69 e	---
Isolate	9.61 ± 0.15 c	6.26 ± 0.40 f	7.72 ± 0.45 g

[1] n = 3
Means followed by different letters are significantly different ($p < 0.05$)

Table 29 Oil Absorption Capacity of Cowpea and Navy Bean Protein Fractions [1]

Fractions	OAC ($g H_2O$ / g protein)		
	Cowpea	Navy Bean	Soybean
Flour	19.80 ± 0.29 a	26.67 ± 0.14 d	---
Extract	16.58 ± 0.07 b,e	18.77 ± 0.61 c,d	---
Isolate	18.61 ± 0.05 c	16.68 ± 0.10 e	7.96 ± 0.11 f

[1] n = 3
Means followed by different letters are significantly different ($p < 0.05$)

Foam capacity (FC) calculated as the percentage volume increase of 5% suspensions of the protein samples is shown in Table 30. It was highest for the navy bean fractions (NBI, NBPE), then SPI, and much lower for CPI. The foam stability over a 6-hour period is illustrated in Figure 17. The decrease in volume was greatest for CF, CHPE and NBF compared to SPI, NBPE and NBI. However, after 6 hrs, the only samples with foams remaining were CF and CPI.

These results can be explained by the properties of a protein for foamability and stability. In NBI, the protein structure is more denatured than in the flour or extract because of shearing during UF processing, and in SPI, unfolding occurred during isoelectric precipitation. This unfolded structure facilitates migration to the interface, and thus the formation of foams. The presence and amount of native rather than denatured proteins have been shown to be related to higher foam stability (Yasumatsu et al, 1972; and Lin et al, 1974). This supports the result that the CF and CPI fractions were the only

Table 30 Foam Capacity of Cowpea and Navy Bean Protein Fractions [1]

Fractions	Foam Capacity (% Volume increase)		
	Cowpea	Navy Bean	Soybean
Flour	10.67 ± 0.16 a	19.33 ± 0.58 d	---
Extract	11.67 ± 0.58 a,b	56.67 ± 3.05 e	---
Isolate	29.00 ± 1.00 c	58.67 ± 1.15 e,f	52.00 ± 2.00 g

[1] n = 3
Means followed by different letters are significantly different ($p < 0.05$)

samples with foams remaining after 6 hrs. The high foam capacity and stability of NBI makes it desirable as an ingredient in whipped food products.

Emulsion capacity (EC) was determined subjectively, and calculated as the ml oil/mg protein (McWatters & Cherry, 1981). EC of all the fractions was higher than the SPI, and decreased with each processing step (Table 31). This supported the results of Okezie & Bello (1988) and McWatters & Cherry (1981) for winged bean and field pea respectively, indicating that as protein content increased, the emulsion capacity decreased. The higher values for the NB fractions and SPI was due to increased denaturation of the proteins in those samples. The emulsion stability of the isolates and SPI (Figure 18) was high, after 38 hrs there was only about a 10-ml separation. This high emulsion stability may be due to the globular nature of the major proteins in legumes, and suggest that the fractions could be used in meat, ice cream, and textured protein products.

Table 31 Emulsion Capacity of Cowpea and Navy Bean Protein Fractions

Fractions	Emulsion (g oil / mg protein)		
	Cowpea	Navy Bean	Soybean
Flour	0.14 ± 0.03 a	0.29 ± 0.02 d	---
Extract	0.13 ± 0.02 b	0.21 ± 0.01 e	---
Isolate	0.11 ± 0.02 c	0.19 ± 0.04 f	0.06 ± 0.03 g

[1] n = 3

Means followed by different letters are significantly different ($p < 0.05$)

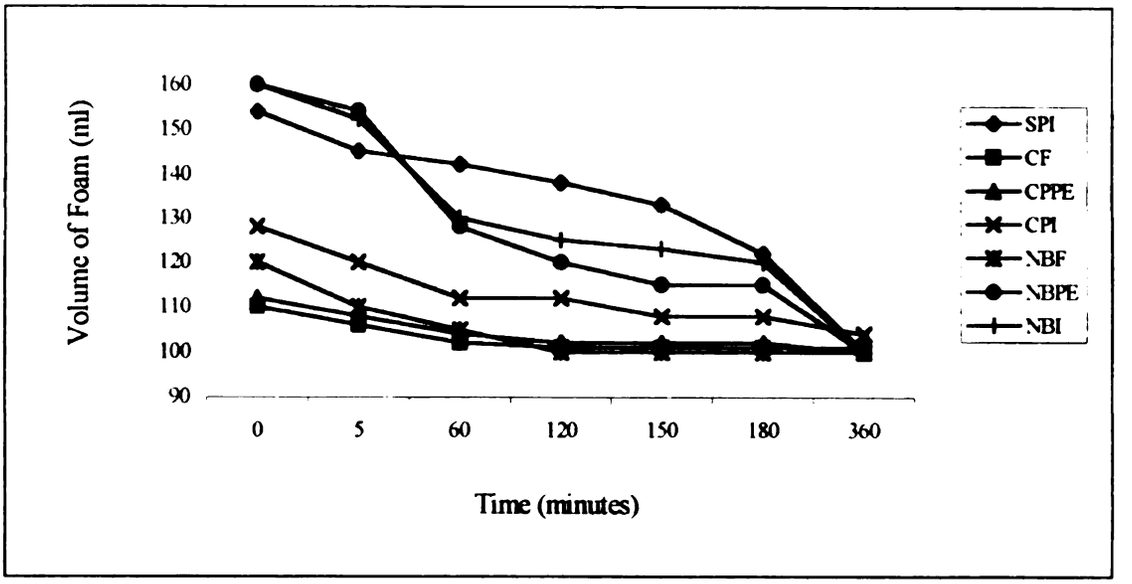

Figure 17 Foam Stability of Cowpea and Navy Bean Protein Fractions

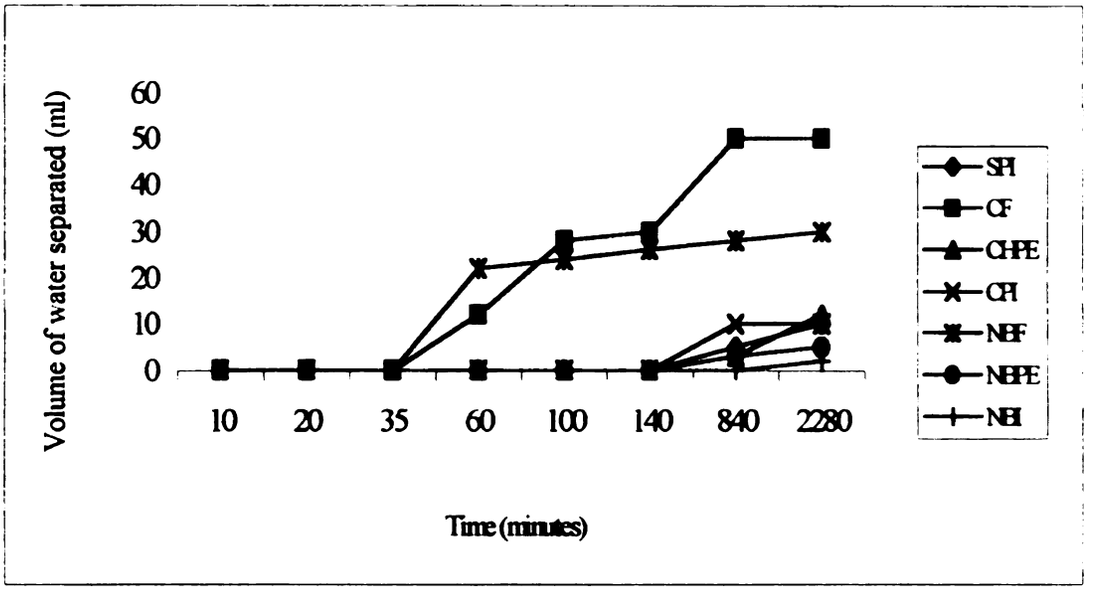

Figure 18 Emulsion Stability of Cowpea and Navy Bean Protein Fractions

Table 32 shows the gelation characteristics of the protein fractions. The critical gelation protein concentrations (% wt/vol.) were 2.66, 6.32 and 9.32 for CF, CPPE and CPI respectively. These were significantly different from the corresponding navy bean fractions which were 1.95, 6.71 and 7.67 for NBF, NBPE and NBI respectively. The critical gelation protein concentration for SPI was 12.27.

Findings of this study indicate that the flour and extract fractions gelled at lower protein concentrations than the isolates, and that the critical protein concentrations (% wt/ml) for gelation increased with increasing protein content. This supports the work of Schmidt (1981), who reported that a high protein concentration is required for the gelation of globular proteins, and that solutions containing both protein and polysaccharides will form gels at relatively low concentrations of the gelling material.

The textural characteristics from an instrumental TPA of 18% gels are also indicated in Table 32. The gel strength determined by 1) the work required to penetrate the gel and 2) gel hardness was highest for the flour fractions than extract or isolate, however, SPI had the highest gel strength compared to the experimental isolate fractions. This supports the least gelation concentration data reported above. In general, the CPI and NBI gels were more adhesive and cohesive, and less chewy and gummy than the SPI gels.

Color reflectance values for the protein fractions are illustrated graphically in Figures 19 - 21. The data indicates that the CP and NB fractions were darker, and had more yellowness and greenness characteristics after extraction and UF processing. SPI was lighter, and had more redness and blueness characteristics than the experimental samples. Analysis of variance indicates that UF processing had a highly significant ($p <$

Table 32 Gelation Characteristics of Cowpea and Navy Bean Protein Fractions after Ultrafiltration Processing [1]

Fractions	Cohesiveness (18% gel) N	Adhesiveness (18% gel) N / mm	Gumminess (18% gel) (N)	Chewiness (18% gel) N / mm	Work Done (18% gel) N / mm	Hardness (18% gel) N	Critical Protein Conc. for Gelation (% wt / ml)
CPF	0.27 ± 0.01 a	1.76 ± 0.05 a	13.01 ± 0.84 a	4.13 ± 0.07 a	37.06 ± 0.64 a	51.82 ± 0.21 a	2.70 ± 0.04 a
CPPE	0.24 ± 0.01 b	1.33 ± 0.05 b	5.69 ± 0.08 b	0.55 ± 0.04 b	18.22 ± 0.04 b	22.99 ± 0.73 b	6.32 ± 0.11 b
CPI	0.38 ± 0.03 c	0.68 ± 0.03 c	7.28 ± 0.15 c	0.75 ± 0.03 c	9.04 ± 0.57 c	20.58 ± 0.39 c	9.30 ± 0.07 c
NBF	0.22 ± 0.03 a,d	1.13 ± 0.09 d	12.88 ± 0.13 a,d	4.55 ± 0.24 d	34.11 ± 0.57 d	44.81 ± 0.68 d	1.92 ± 0.03 d
NBPE	0.28 ± 0.02 e	0.99 ± 0.02 e	4.54 ± 0.30 e	0.39 ± 0.05 b,e	24.79 ± 0.39 e	21.60 ± 0.77 c,b,e	6.73 ± 0.08 e
NBI	0.39 ± 0.01 b,f	0.76 ± 0.05 c,f	6.05 ± 0.17 b,f	0.46 ± 0.04 b,e,f	7.09 ± 0.13 c,f	19.36 ± 0.46 c,f	7.74 ± 0.13 f
SPI	0.27 ± 0.02 g	0.23 ± 0.02 g	11.57 ± 0.12 g	2.18 ± 0.11 g	12.09 ± 0.38 g	42.50 ± 0.55 g	12.68 ± 0.35 g

[1] n = 3

Means within the same column followed by different letters are significantly different ($p < 0.05$)

0.0001) effect on the color of the fractions. In addition, all of the fractions were significantly different from SPI, the control. Xu and Diosady (1994) and Tjahjadi et al (1988) observed this darkening color for chinese rapeseed and adzuki bean proteins, respectively after extraction and isolation.

The F-values of all functional properties and their statistical significance indicate that extraction and UF processing had a significant effect on the functional properties of the fractions. There was also a significant difference in the functional properties of the experimental fractions and SPI.

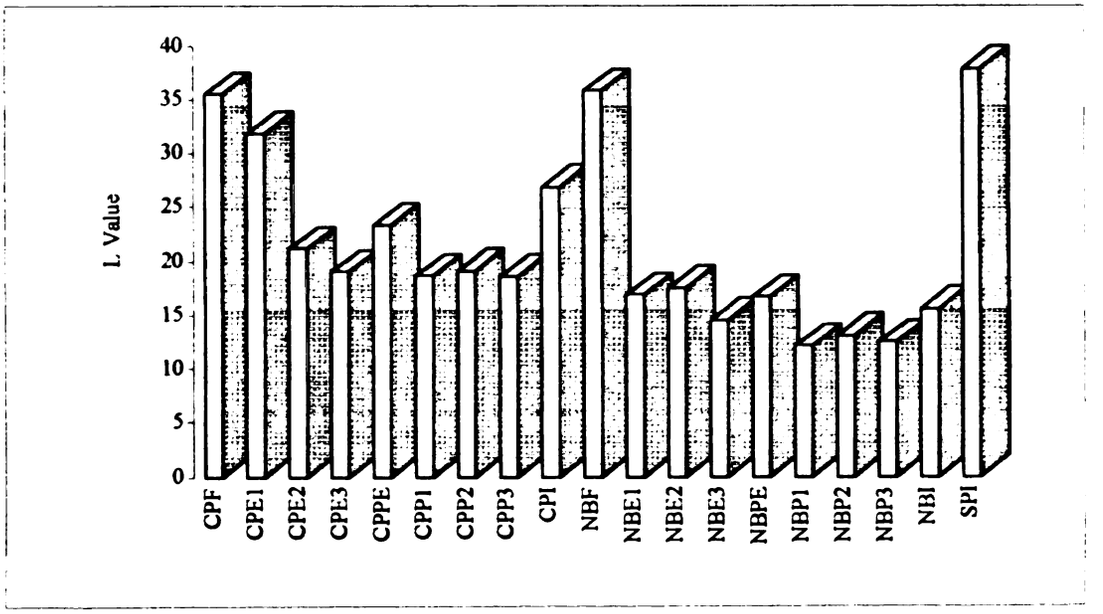

Figure 19 Hunter Lab L-value (Lightness) of Cowpea and Navy Bean Protein Fractions after UF Processing

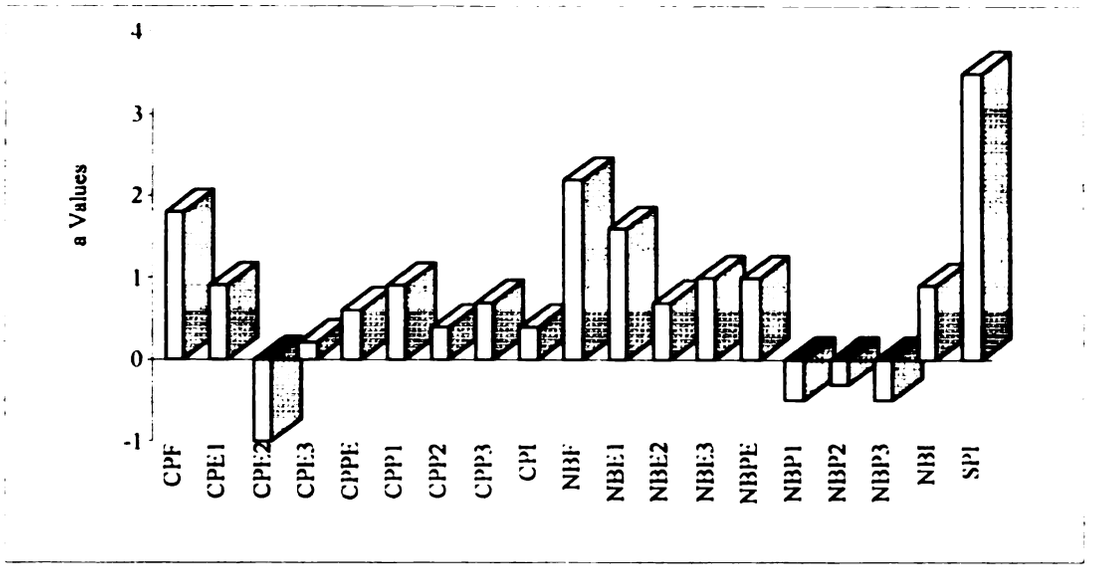

Figure 20 Hunter Lab a-Value (redness-greeness characteristics) of Cowpea and Navy Bean Protein Fractions after UF Processing

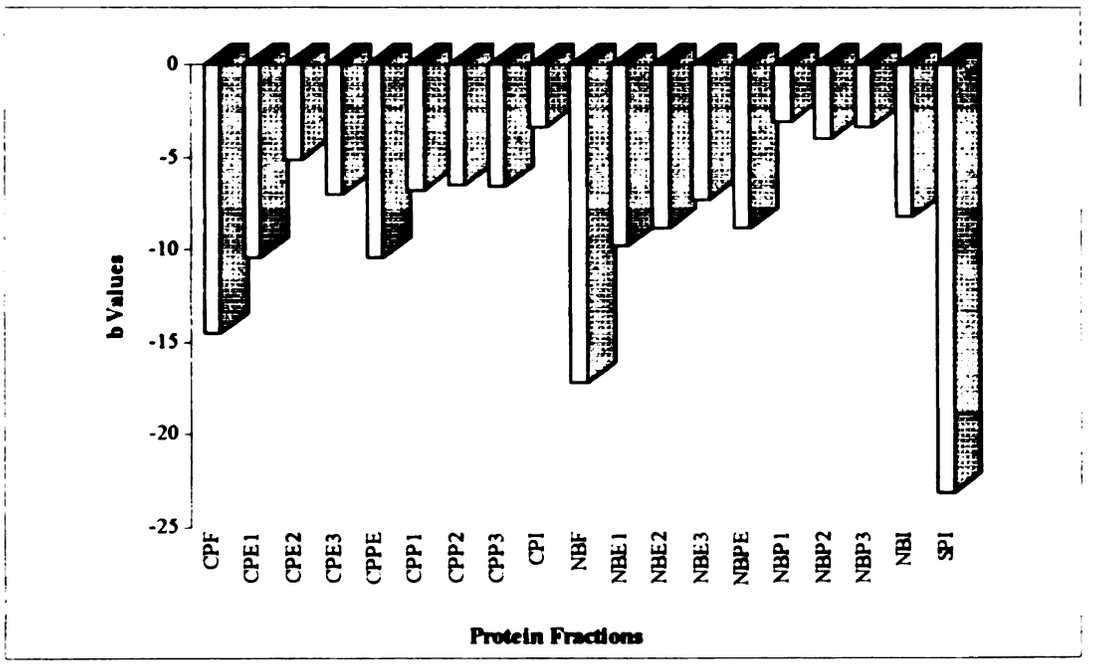

Figure 21 Hunter Lab b-Values (yellowness-blueness characteristics) of Cowpea and Navy Bean Protein Fractions after UF Processing

The correlation matrix of the functional properties of cowpea, navy bean and soybean protein fractions indicated that EC, OAC, GEL P, and ADHES were highly correlated to %P of the fractions, while FC and WORK had mid-range correlations. All had negative correlations to %P except FC. HARD and GUM were highly correlated to all color values of the fractions, while chewiness had mid-range correlations. High to mid-range correlations was found between WORK and the other TPA textural characteristics, and mid to low-range correlations found with FC, EC and OAC. All of these correlations were positive except FC, GEL P and COHES. OAC had high positive and negative correlations respectively to EC and GEL P, while WAC had only a mid-range negative correlation with ADHES. PS showed positive correlations with COHES and B color value.

The correlation matrices of the functional properties of cowpea and navy bean protein fractions grouped by bean type indicated that %P in CP fractions showed high to mid-range correlations with all properties except OAC, while %P for NB had high correlations with all properties. These results indicate that the protein content and hence UF processing was important in functionality of the cowpea and navy bean protein fractions.

Factor Analysis in Stat View (SAS Institute Inc.) was used to determine which of the functional properties was most affected by ultrafiltration processing, that is, which were the principal components. The factors were then transformed by a varimax orthogonal rotation to achieve a more meaningful interpretation. The number of factors retained was determined by those factors having eigenvalues equal to or greater than one, and functional properties with loadings between 0.70 and 0.99 were considered major.

The number of variables were fifteen, and an estimated seven factors were extracted. Of these, four had eigenvalues greater than one, and they accounted for about 95% of the variance observed. Table 33 indicated that CHEW, HARD, WORK and GUM exhibited the highest loading in factor I, which accounted for 44% of the total variance observed. These properties were all characteristics of the legume protein gels, thus factor I can be called a "gelation" factor. The correlation matrix of the functional properties of cowpea and navy bean protein fractions indicated high positive correlations between CHEW, HARD, WORK and GUM.

OAC, ADHES, GEL P, and Hunter "b" had the highest loading on factor II, which accounted for 27% of the total variance observed. These variables represent a mixture of several properties including hydration, gelation and color. However, the correlation matrix indicated low correlations between hunter color characteristics and OAC or GEL P. Thus Factor II can be characterized as "hydration-gelation." Table 33 indicated that the loading coefficients for OAC and ADHES were positive, while that for GEL P was negative. This supported the high negative correlation observed between OAC and GEL P, and ADHES and GEL P; and the high positive correlation observed for OAC and ADHES.

WAC was the only variable that expressed a high loading coefficient in factor III, which accounted for 12% of the variance observed. Since WAC is a hydration functional property, factor III can thus be characterized as a "hydration" factor. EC, FC and PS showed the highest loading coefficients in Factor IV, which accounted for 11% of the total variance observed. These variables have both surface property and hydration in common, and could be characterized as a "surface property + hydration" factor.

In conclusion, the results of the factor analysis suggested that the four factors extracted from the functionality study were "gelation", "hydration + gelation", "hydration" and "surface property + hydration." Thus, gelation, hydration and surface property characteristics can be considered as the principle functional properties that are affected by ultrafiltration processing of legume proteins.

Table 33 Factor Analysis Loading of 15 Functional Properties on the first four factors (eigenvalues > 1) after Varimax rotation [1]

Functionality	Factor 1	Factor 2	Factor 3	Factor 4
FC	- 0.593	-0.451	0.127	0.512
EC	0.290	0.595	0.305	0.659
WAC	-0.202	-0.366	0.809	0.266
OAC	0.386	0.777	0.400	0.246
PS	-0.396	0.370	0.504	-0.639
GEL P	-0.637	-0.750	-0.068	-0.108
WORK	0.864	0.375	-0.069	0.151
HARD	0.919	-0.275	0.095	-0.177
ADHES	0.506	0.761	-0.285	-0.208
CHEW	0.944	-0.040	0.300	-0.086
GUM	0.851	-0.314	0.321	-0.245
COHES	-0.768	0.057	0.475	-0.268
Hunter "L"	0.784	-0.442	0.186	-0.326
Hunter "a"	0.606	-0.737	0.069	0.231
Hunter "b"	-0.674	0.681	0.163	-0.126
Eigenvalue	6.67	4.10	1.76	1.66
Variance (%)	44.5	27.3	11.7	11.1
Cumulative Variance (%)	44.5	71.8	83.5	94.6

[1] Loading values > 0.70 are underlined and considered significant

Table 34 summarizes the results of an assay for screening phytohemagglutinin (Lectin) activity. It indicates that significant lectin activity was observed in flour and extract fractions for all samples, but less so for isolates. High activities were also observed in the residue and permeate 3 fractions. These results are consistent with UF processing reducing lectin activity in the isolates. This was supported by the data available on the doublet band size of phytohemagglutinin (32KD and 31KD) by Coffey, (1983). In this study, permeates 2 and 3 corresponding to the UF membranes with MWCO of 15-30KD and 50-100KD recorded the highest lectin activities, which suggests that lectin permeated those UF membranes and accumulated in the permeates.

Table 34 Screening Cowpea and Navy Bean Protein Fractions for Phytohaemaglutinins (Lectins) [1]

Fractions	Activity observed after 1 hour		
	Cowpea	Navy Bean	Soy Bean
Flour	++++	++++	++++
Extract	+++	+++	---
Residue	+++	+++	---
Permeate 1	+	+	---
Permeate 2	+	+	---
Permeate 3	++	++	---
Isolate	+	++	+

[1] n= 3
+ indicates the presence of a precipitate
number of + indicates degree of precipitation
--- indicates sample not analyzed

The data on *in-vitro* digestibility is shown in Table 35. It indicates that for both cowpeas and navy beans, digestibility increased after aqueous extraction of flour and UF processing of the extract. Digestibility of cowpea fractions was higher than navy beans for the two in-vitro methods tested, however digestibility of CPI and NBI were both lower than that for SPI. This was expected since the unfolded state of SPI permitted easier digestion. A highly significant difference in treatments was observed for both methods ($p < 0.001$).

Table 35 *In-vitro* Protein Digestibility of Cowpea and Navy Bean Fractions after Ultrafiltration Processing

Fractions	pH-Stat	pH-Drop
CPF	81.29 ± 0.38 a,d,e,f	68.49 ± 1.49 a
CPPE	82.87 ± 0.49 a,b	77.23 ± 1.86 b,e
CPI	82.68 ± 0.62 a,b,c	75.37 ± 1.43 b,c
NBF	79.38 ± 0.82 d	75.68 ± 1.07 b,d
NBPE	79.37 ± 0.98 d,e	79.03 ± 0.89 e
NBI	81.69 ± 0.40 b,c,f	80.99 ± 0.82 e,f
SPI	90.11 ± 0.21 g	94.32 ± 1.14 g

[1] n = 3
Means within the same column followed by different letters are significantly different ($p < 0.05$)

Interpretation of the *in-vitro* digestibility for fractions that had extremely low protein content (permeates) and possibly low digestibility was difficult by the pH-Stat assay. The digestibilities indicated for cowpea and navy bean permeate and residue fractions may have been overestimated using this method. None of these fractions

required any additional NaOH to maintain the pH at 7.98, hence based on the equation used, digestibility was calculated as 79.28%. The equation however assumed that the minimum digestibility would be 79.28%, but this may not be case with those samples.

There are limitations with using derived equations in determining *in-vitro* digestibility. The 3 enzyme *in-vitro* method described by Hsu et al (1977) was reported to underestimate protein digestibility particularly from animal sources (Hsu et al, 1978). This was modified by Satterlee et al (1979) who introduced a four enzyme method, however it was concluded that only approximate estimates of protein digestibility was possible with these procedures (Bodwell et al, 1980). It was reported that the three enzyme pH-Stat method provided more accurate estimates of digestibility because the activity of the enzymes used was pH dependent. Since pH was kept constant during digestion, this ensured more uniform enzyme activity and thus more accuracy than with the pH Drop method (McDonough et al, 1990).

However, in a collaborative test by McDonough et al (1990) in which the pH-Stat method was used, they reported average digestibilities of 99% for soy isolate, 97.6% for pea concentrate, 93.8% for canned chick peas and 93.7% for canned pinto beans. These digestibility values are extremely high for legume products. They are significantly higher than what is reported in this study for cowpeas and navy beans; and in other studies of legume protein digestibility (Rubio et al, 1991; Eicher and Satterlee, 1988; Rozan et al, 1997; Kohnhorst et al, 1990 and Hernandez et al, 1997). Despite the problems with using both methods, they produced comparable results for estimates of protein digestibility. The two *in-vitro* digestibility assays showed a high positive correlation (0.74), and mid-

range negative correlations with lectin activity, that is, pH Stat vs. lectin, r = – 0.66 and pH Drop vs. lectin, r = – 0.64.

Conclusion

Results of this study indicated that UF processing was effective at separating proteins on the basis of their size. The physico-chemical properties of cowpea and navy bean proteins were significantly affected by UF processing, and there were significant differences between the experimental fractions and SPI. Protein and amino acid composition of both legume fractions increased significantly. The limiting amino acids in the fractions were the sulfur amino acids, methionine and cysteine.

The fractions underwent denaturation from shearing during UF processing; cowpea fractions appeared to be less denatured than navy bean, while SPI was more denatured than both cowpea and navy bean fractions. The denatured state of the protein fractions affected protein functionality, particularly protein solubility of the fractions. SPI, with the greatest unfolded structure, had the lowest protein solubility, which would affect its use in commercial hydration applications. Protein digestibility determined by *in-vitro* assays, significantly improved after UF processing, which was associated with reduced lectin activity, and increased protein and amino acid content.

The high PS of CPI and NBI indicate that these fractions would have better hydration properties than SPI, and would be useful in liquid applications. Additionally, the high WAC of CPI compared to SPI makes this fraction better than SPI for liquid products. The high FC and FS of NBI makes it better than SPI in whipped products such

as cakes, desserts and ice cream. OAC and EC of CPI and NBI were higher than SPI, and ES highest in NBI, these make the experimental isolates, particularly NBI, better in simulated meat products, cakes and dressings than SPI. Critical protein required for gelation was highest for SPI, which results in the experimental isolates being better in protein gels such as meats, cakes and cheese products.

Therefore, the null hypothesis (H_o), stated as there are no significant differences between the physico-chemical properties of cowpea and navy bean protein fractions compared to a commercial SPI after UF processing, must be rejected. The conclusion from this study is that UF processing influences the physico-chemical properties of proteins.

Future Research

The goals for future study include the following:

1. Optimize protein extraction and improve membrane performance by introducing a continuous slurry centrifugation operation and examine its effect on protein yield
2. Further research on the structure of individual proteins in cowpeas and navy beans is necessary to explain the differences observed in flux measurements between the two during UF processing
3. Expand characterization studies on permeates to determine its composition and utilization in commercial applications

STUDY 3 NUTRITIONAL QUALITY OF COWPEA AND NAVY BEAN PROTEIN DIETS BY *IN VITRO* AND *IN VIVO* PROTEIN DIGESTIBILITY CORRECTED AMINO ACID SCORE (PDCAAS)

Abstract

The nutritional value of the insoluble residue that remains after aqueous alkali extraction of legume flours requires additional research effort to assess its potential as a food ingredient, particularly in countries where protein-energy malnutrition persists. The protein digestibility of cowpea (CP) and navy bean (NB) residue diets were studied by *in vivo* and *in vitro* assays. The legume residues were cooked then air-dried prior to diet preparation. All diets contained 10% protein (w/w). Legume proteins supplied 30%, 70% and 100% of the protein; the remainder was derived from wheat flour. A modified American Institute of Nutrition (AIN) rodent diet AIN-93G was used as the control and a 2% albumin diet was used to determine metabolic fecal nitrogen. Food intake and body weight of the rats was measured and apparent and true protein digestibilities calculated for the *in vivo* study. The pH stat and pH drop *in vitro* assays were also used to determine protein digestibility. Amino acid composition for all diets were measured and *in-vivo* and *in-vitro* protein digestibility corrected amino acid scores (PDCAAS) calculated. The food intake of all CP diets was greater than that observed for the control, while that for all NB diets was less. Intake of the 30% and 70% CP and NB diets was not significantly different from each other. The 100% CP and NB diets were consumed the least. All diets supported rat growth except 100% NB. All rats fed CP diets had significantly higher weights than the control, unlike rats fed NB diets which all had lower weights. *In-vivo* digestibility ranged

from 73.7% - 87.5% and 62.6% - 78.2% for CP and NB diets respectively, compared to 98.1% for the control. All diets had significantly different digestibilities than the control except 30% CP. The 70% CP, 100% CP and NB diets were not significantly different from each other. Significant correlation's ($p < 0.05$) were 0.73 for pH stat vs pH drop, 0.86 for pH stat vs *in vivo* and 0.89 for pH drop vs *in vivo*. Amino acids that are generally limiting in legumes increased in concentration after supplementation with wheat flour. The 100%CP, 100%NB and 30%NB diets were limiting in sulfur amino acids and lysine respectively, and were the only diets that did not meet the suggested pattern of requirement for pre-school children (2-5yrs). Lysine (Lys) was lowest in the 30%CP and 70%NB diets; methionine + cysteine (Met + Cys) was lowest in 70%CP; and threonine (Thr) was lowest in the modified AIN-93G. *In-vivo* and *in-vitro* assays for PDCAAS was based on these limiting amino acids, and were highly correlated ($r = 0.94$, $r = 0.97$ and $r = 0.98$, $p < 0.01$). These results suggest that *in-vivo* and *in-vitro* PDCAAS could accurately predict protein nutritional quality. Additionally, the insoluble CP and NB residues could not be recommended nutritionally as food for pre-school children, but could be recommended if they are supplemented with wheat flour.

Introduction

The quality of a protein depends on the kinds and amounts of amino acids it contains, and represents a measure of the efficiency with which the body can utilize the protein. A balanced or high quality protein contains essential amino acids in sufficient ratios for human needs. Proteins of animal origin generally tend to be of higher quality than those of plant origin. Amino acids present in dietary proteins are not necessarily fully "available" since digestion of the protein or absorption of the amino acids may be incomplete. Amino acids from animal foods are absorbed to an extent of 90%, while those from plant foods are digested and absorbed to an extent of only 60-70%. This has been attributed to the protein conformation; binding to metals, lipids, cellulose and other polysaccharides; the presence of antinutritional factors such as trypsin inhibitors and lectins; as well as surface area and size of the protein, processing effects and biological differences amongst individuals (FAO, 1970, 1973).

The nutritional quality of legume proteins has been extensively reported in the literature. Bressani (1973) showed that most legume proteins had low biological value, ranging from 32-78%, because of the low concentration of sulfur amino acids in legume protein. Kelly (1973) showed the beneficial effects of the addition of methionine in the diet when legumes are used as the protein source; he found that both the protein efficiency ratio (PER), and the average weight increased. However this was not evident for all legume species, and was explained on the basis that methionine is not the most or the only limiting amino acid in legume species. When both methionine and tryptophan were added, protein quality increased which indicated that both are limiting.

More recently, significant research has begun to be expended on other leguminous species, as their desirability as less expensive protein sources becomes obvious, particularly to nations where protein-energy malnutrition was prevalent. Ulloa et al (1988) obtained a protein concentrate from chickpea (Cicer arietinum) by UF membranes and used it in infant formulas. Bolles (1997), reported on the nutritional quality of a navy bean retentate used in an aseptically processed infant product and found that it was of similar quality and functionality as a soy protein isolate.

However, there has been very little work reported on the nutritional quality of the residue that remained after aqueous alkali extraction, and its use as a food ingredient. Preliminary results from this work have indicated that the protein content of the residue ranges between 18 - 25% after extraction, and about 35% of the total available protein remained in the residue (Jackson et al, 1997). Additionally, sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) analysis from an earlier study indicated similarity in protein bands between the residue and flour. Both flour and residue also showed similar amino acid profiles. Opportunities therefore exist to evaluate the applicability of the residue as a food ingredient. Six diets that contained only cowpea residue and navy bean residue, a mixture of wheat and the cowpea or navy bean residue, and two other diets containing 2% albumin and a control modified AIN-93G diet were used. All diets contained about 10% protein and arrowroot starch as their carbohydrate source.

The objectives of this study were 1) to determine the effect of diet type on food consumption and rat growth, and 2) to estimate the protein quality of the diet blends.

The null hypotheses (H_o) being tested is there are no significant differences in protein quality of the experimental diets compared to the control modified AIN-93G diet.

Materials & Methods

The raw residue that remained after aqueous alkali extraction of cowpea and navy bean flour was cooked in kettles to inactivate antinutritional factors, then air-dried for 48 hours at 45°C. They were stored at 4°C prior to use.

Experimental diets were prepared based on a 10% protein diet, using a modified AIN-93G diet as the control. Actual protein content of each diet was determined by the micro-kjeldahl method (AACC, 1984), using a nitrogen to protein factor of 5.7 for the legume diets and 6.25 for the modified AIN-93G and 2%ALB diets. Proteins were hydrolyzed with HCl to determine total amino acid composition of each diet, except tryptophan and cysteine. Methane sulfonic acid (MSA) hydrolysis was used for tryptophan analysis and formic acid oxidation followed by acid hydrolysis for cysteine analysis.

In-vitro digestibilities of the experimental diets were measured using the three-enzyme pH Stat assay (McDonough et al, 1990), and the four-enzyme pH Drop assay (AOAC 1990). Digestibilities of the diets were calculated using the following equations:

pH Drop % Protein Digestibility = 234.84 - 22.56 (X), where X = pH at 20 min

pH Stat TD = 76.14 + 47.77B, where B = ml 0.1N NaOH added

Twenty weanling male Sprague-Dawley rats (Harlan Sprague Dawley Inc., MI) were used in the *in-vivo* feeding study over a period of 4 weeks. The rats were ranked by weight and assigned diets so that none of them received the same diet twice. Each diet

was fed to groups of ten rats in a completely randomized design. Rats were housed in individual cages and given free access to diets and water for 7 days. The animals were weighed daily, food intake was measured, and spilled food and fecal matter collected, air-dried and weighed between days 3 and 7. Fecal samples were ground in a mortar and stored at 4°C prior to analyses. Apparent and true digestibilities were calculated.

Protein quality of the experimental diets was estimated using relative protein efficiency ratio (RPER), relative net protein ratio (RNPR), relative true protein digestibility (RTD), and *in-vivo* and *in-vitro* protein digestibility corrected amino acid score (PDCAAS).

Statistical Analysis

Microsoft Excel was used to compute the averages and standard deviations of the variables. Stat View (SAS Institute Inc., 1998) was used to analyze the food intake, rat growth and digestibility data, and determine differences between diets using Fisher's LSD. Correlation matrices of protein nutritional quality tests for the diets were generated.

Results and Discussion

The composition of a two-kg sample of each experimental diet is shown in Table 36. The protein contents of the whole-wheat flour, navy bean and cowpea residues were 11.03%, 15.25% and 14.27% respectively. Diets 1 - 6 contained a combination of 30%, 70%, 100% navy bean or cowpea protein supplemented with 70%, 30%, 0% wheat flour respectively. Diet 7 was 2% albumin to estimate metabolic fecal nitrogen and diet 8 was the control modified AIN-93G diet with casein as the protein source. The major carbohydrate source for all diets was arrowroot starch, which ranged in weight from 0.01 - 71% of the total diet weight.

Table 37 shows the actual protein content and standard deviation of each diet. Protein content ranged from 9.08 – 11.78% indicating that none of the samples contained 10% protein. The protein contents were within the range of values reported by Sarwar (1997) for use in *in-vivo* assays. CP diets had higher protein contents than the NB diets, 100% CP had the highest protein content of 11.78% while 30% NB had the lowest protein content of 9.08%. The actual protein content of the diets were highly significantly different ($p < 0.0001$) from each other. All of the NB diets were not significantly different from the each other, however, only 30%CP and 100%CP diets were not different. The 70%CP, 30%NB, 70%NB, and 100%NB were not significantly different from the control, modified AIN-93G diet.

Table 36 Cowpea and Navy Bean Protein Diet Composition

Ingredients	70%NB	70%CP	30%NB	30%CP	100%NB	100%CP	2%ALB	M-AIN-93G
WWF	543.97	543.97	1269.27	1269.27				
NBR	918.03		393.44		1311.47			
CPR		981.08		420.46		1401.54		
albumin							2.00	
casein								
corn oil	214.94	214.94	214.94	214.94	214.94	214.94	214.94	429.88
fiber	20.68	9.33	4.87		32.55	16.33	268.61	537.22
mineral mix	70.00	70.00	70.00	70.00	70.00	70.00	70.00	140.00
vitamin mix	20.00	20.00	20.00	20.00	20.00	20.00	20.00	40.00
CB	5.00	5.00	5.00	5.00	5.00	5.00	5.00	10.00
BHT	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.06
Arrowroot	207.35	155.65	22.46	0.31	346.01	272.16	1419.42	2842.84
Total	2000	2000	2000	2000	2000	2000	2000	4000

Table 37 Actual protein content and standard deviation of the experimental and modified AIN-93G diets [1]

Diet	Protein content (%)
30% CP	11.66 ± 0.35 a
70% CP	9.63 ± 0.45 b,h
100% CP	11.78 ± 0.47 a,c
30% NB	9.08 ± 0.58 b,d,h
70% NB	9.76 ± 0.24 b,d,e,h
100% NB	9.42 ± 0.29 b,d,e,f,h
2% Albumin	2.35 ± 0.37 g
AIN-93G	9.26 ± 0.27 h

[1] n = 3
means with the same letters are not significantly different ($p < 0.0001$)

Chromatograms of the acid hydrolyzed amino acid profile of each diet indicated that the peaks of all diets were well resolved and showed a similar profile as CPF and NBF fractions. The average peak areas and retention times for amino acids in the experimental diets indicated similar retention times to the amino acid standard solution (Sigma A9781).

The amino acid composition of the residues and diets is shown in Table 38. The cooked residues had lower concentrations of amino acids than the uncooked residue. The lower concentration was due to the processing treatment that the cowpea and navy bean residues received during cooking and drying prior to diet preparation. The amino acid content of the experimental diets were significantly different from each other ($p < 0.05$).

Table 38 Amino Acid Composition of Cowpea and Navy Bean Diets (mg amino acid / g protein)

Amino Acid	30CP	70CP	100CP	30NB	70NB	100NB	M-AIN-93G
Asp	12.33	19.76	20.72	13.55	9.59	12.06	12.85
Glu	32.57	40.20	28.83	36.14	21.55	17.24	35.13
Ser	6.79	10.76	8.61	7.35	7.32	7.38	9.49
Gly	7.10	11.94	6.53	7.23	5.05	7.43	4.71
His	6.54	11.36	4.00	6.15	2.04	5.40	5.01
Arg	5.99	10.47	10.73	6.52	5.85	7.46	9.12
Thr	7.73	13.37	6.18	8.29	4.65	8.59	7.01
Ala	0.68	1.43	8.20	ND	5.60	0.80	0.53
Pro	9.37	10.80	5.69	9.88	6.16	4.81	14.71
Tyr	3.32	5.35	4.26	3.30	2.89	2.82	6.18
Val	6.63	11.53	10.78	7.61	6.53	7.23	11.19
Met	4.63	3.20	2.28	3.70	1.46	1.42	4.21
Cys[1]	4.90	2.97	1.57	4.67	4.61	2.57	17.21
Ile	5.86	10.06	8.94	6.52	5.64	6.44	9.33
Leu	9.71	16.50	13.80	10.29	9.13	10.01	15.23
Phe	8.34	14.39	12.19	8.50	7.55	8.47	10.07
Trp[2]	5.89	5.22	1.49	6.64	3.75	2.95	11.40
Lys	5.79	12.58	12.09	5.86	6.65	8.40	12.82

[1] Cys analysis by formic oxidation and acid hydrolysis
[2] Trp analysis by MSA hydrolysis

All diets were richest in glutamic and aspartic acids. The concentrations of cysteine, methionine and tryptophan, the limiting amino acids in legumes, increased after complementation with wheat protein in the diets. As expected, diets that had the highest wheat protein, that is, 30% and 70% diets had the lowest levels of lysine than the other diets. Similarly, diets that had the highest legume protein, that is, 100%CP and 100%NB diets had lower levels of sulfur amino acids and tryptophan than those with greater wheat protein. The modified AIN-93G diet was significantly different from all experimental diets and had high amounts of the amino acids that were limiting in the legume diets.

Table 39 shows the amino acid score of the experimental diets based on the reference amino acid pattern for pre-school children (2 – 5 yr.) (FAO/WHO, 1990). It indicates that only the 100%diets and 30%NB did not satisfy the reference amino acid pattern for pre-school children. Met + Cys were the limiting amino acids in the 100% diets and Lys in the 30%NB diet. All of the other diets satisfied the amino acid requirements for pre-school children. This data is consistent with what has been reported in the literature for lysine and the sulfur amino acids, as the limiting amino acids in wheat and legume protein sources respectively (FAO/WHO, 1973).

Phytohemagglutinin (Lectin) activity of the cooked and uncooked residues and each diet is shown in Table 40. It indicates that lectin activity was only observed in the raw cowpea and navy bean residue samples, CPR and NBR respectively. There was no detectable lectin activity in the cooked samples after receiving heat treatments during cooking and air-drying. Similarly, none of the experimental diets or the control diet

Table 39 Essential Amino Acid Score for Cowpea and Navy Bean Diets[1]

Amino Acid	30CP	70CP	100CP	30NB	70NB	100NB	M-AIN-93G
Cys + Met	1.94	1.29[2]	0.78	0.96	1.36	0.62	2.28
Trp	3.75	2.92	1.75	3.67	2.50	2.33	2.28
Lys	1.02	2.03	1.95	0.95	1.07	1.36	2.07
Thr	2.15	3.71	1.72	2.30	1.29	2.39	1.95
Phe + Tyr	1.77	2.99	2.49	1.79	1.58	1.71	2.46
His	3.27	5.68	2.00	3.07	1.02	2.70	2.50
Ile	1.95	3.35	2.98	2.17	1.88	2.15	3.11
Leu	1.39	2.36	1.97	1.47	1.30	1.43	2.18
Val	1.79	3.12	2.91	2.06	1.76	1.95	3.02

[1] Based on the FAO/WHO/UNU (1985) pattern of amino acid requirements for pre-school children (2 – 5 yr.)
[2] Underlining denotes the lowest amino acid score

displayed any lectin activity. Occena (1994), Coffey et al (1992) and Dhurandar and Chang (1990) also reported on lectin activity. They found that wet heat was most effective in inactivating lectins, and a heat treatment of 100°C for 10 min was sufficient to inactivate all lectin activity in navy beans.

Table 40 Relative Lectin Activity of Residues and Experimental Diets [1]

Diets	Relative Lectin Activity	
	CP	NB
30%	ND	ND
70%	ND	ND
100%	ND	ND
Raw Residue	+++	+++
Cooked Residue	ND	ND

[1] ND - not detectable
Activity in modified AIN-93G diet had ND

In-vitro protein digestibilities determined by pH Stat and pH Drop methods are shown in Table 41. The average values for CP diets ranged from 80.30% - 84.54% and 77.82% - 79.93% for the pH stat and pH drop methods respectively. The average values for NB diets ranged from 79.28% - 79.59% and 66.51% - 74.89% for the pH stat and pH drop methods respectively. All of the CP diets had higher digestibilities than the NB diets using both *in-vitro* assays. A similar trend was observed for the digestibilities obtained by the two methods in all samples. As legume protein concentration in the diet increased,

digestibility decreased for all diets. That is, the 30% CP and 30% NB diets had the highest digestibility of each of their groups with both assays.

Statistical data (Table 41) indicated that there was a highly significant difference ($p < 0.0001$) in digestibility between the diets using both assays. Fisher's LSD indicated that all of the experimental diets were significantly different from the control AIN-93G diet. The digestibility of the navy bean diets did not appear to be significantly different from each other, however the digestibility of the 30% and 70%CP diets appeared to be more similar than the 100%CP diet. This suggests that supplementation with wheat flour had a significant effect on *in-vitro* digestibility, and is related to the increase in essential amino acid composition, particularly the sulfur amino acids, that were limiting in the residue.

The improvement in *in-vitro* protein digestibility of cereal supplemented legume based diets was also reported by Dominguez et al (1993) for 70% press-dried millet and 30% press-dried cowpea diet blend. Wolzak et al, (1981) reported on the *in-vitro* protein digestibility of various cereal-legume blends. They found that a 70:30 ratio of cereal to legume, and rice rather than maize supplemented with black beans gave higher *in-vitro* protein digestibilities. This was possibly due to the range of amino acids present with these combination of foods.

Table 41 also shows that $R = 0.98$ for both pH Stat and pH Drop, which indicates that there was a good fit between diet type and *in-vitro* protein digestibility for both assays. The values of 0.97 and 0.96 for R^2 of pH Stat and pH Drop respectively, indicated that 97% and 96% of the variance in protein digestibility was predictable from the model. Although lower values were observed for protein digestibility using the pH Drop method,

there was a highly significant correlation, r = 0.78 ($p < 0.0001$), between the two methods. This significant correlation for pH Drop and pH Stat methods was also reported by El and Kavas (1996) also observed for digestibility of rainbow trout.

Table 41 *In-vitro* Digestibility [1] and Statistical Analyses [2] of Diets

Diets	In-vitro Digestibility	
	pH-Stat	pH-Drop
30%CP	84.54 ± 0.83 a	79.93 ± 0.42 a
70%CP	82.54 ± 0.87 a,b	77.97 ± 0.84 a,b
100%CP	80.30 ± 0.28 c	77.82 ± 0.04 a,b,c
30%NB	79.59 ± 0.32 c,d	74.89 ± 0.94 b,c,d
70%NB	79.55 ± 0.38 c,d,e	68.77 ± 0.31 e
100%NB	79.28 ± 0.04 c,d,e,f	66.51 ± 0.11 e,f
Modified AIN-93G	93.72 ± 0.71 g	84.92 ± 0.74 g
	Statistics	
F Ratio	51.70	28.02
R	0.98	0.98
R^2	0.97	0.96

[1] n = 3

[2] Means within a column followed by the same letters are not significantly different ($p < 0.0001$)
pH Stat vs. pH Drop, r = 0.78

The raw data on food intake for each rat during the four feeding indicates that there were fluctuations in the quantity of food ingested by each rat, which is related to the type of diet ingested. The average food intake and standard deviation for each diet over the feeding period is indicated in Table 42. It ranged from 27.1 g for 100%NB diet to 75.6 g for 30%CP diet. The food intake of all CP diets was greater than that observed for all NB diets and the modified AIN-93G diet. However, food intake for all NB diets was less than the modified AIN-93G diet. The food intake of the 2% Albumin (2%Alb) diet was less than all diets except 100% NB.

Regression was used to investigate the effect of diet on food intake. Diet had a highly significant effect ($p < 0.0001$) on food intake, and both diet and food intake were highly correlated, $R = 0.86$ and $R^2 = 0.74$. Fisher's LSD indicated that the food intake of all experimental diets were significantly different from the modified AIN-93G diet except 100%CP and 70%NB. Food intake of the 30% and 70% diets were not significantly different from each other for both CP and NB diets. Food intake of 100%NB and 2%Alb were significantly different from all other diets.

The data on rat weight for each group indicates that the final weight range for group 1 rats is 87 – 142 g, group 2 is 94 – 174.8 g, group 3 is 87 – 175 g and group 4 is 118.4 – 189.3 g. The rat growth according to diet is shown in Table 42. It indicates that all CP diets, 30%NB, 70%NB and modified AIN-93G supported rat growth. As expected, the rats fed 2%Alb decreased in weight, as well as those fed 100%NB diet.

The data suggests that when NB or CP residue was the major source of protein, the growth of rats was impaired. This was due primarily to the reduced quantity of essential amino acids in these diets. A regression analysis indicates that diet type had a

highly significant effect ($p < 0.0001$) on rat growth, and diet and rat growth were highly correlated, $R = 0.89$ and $R^2 = 0.79$. The change in rat growth and food intake with diet is presented in Figure 22 and shows that when food intake decreased, rat growth also decreased.

Regression analysis of the effect of diet type, food intake and their interaction on rat growth indicates that both diet and food intake had a significant effect ($p < 0.02$ and $p < 0.0001$ respectively), while the interaction had no effect on rat growth. The multiple correlation coefficient $R = 0.93$, $R^2 = 0.86$ and the lack of fit is not significant. This suggests that the model is able to adequately predict rat growth.

Table 42 Average Food Intake[1] and Standard Deviation of the Experimental Diets and Rat Growth[2] over the Feeding Period

Diets	Food intake (g)	Rat Growth (g)
30 CP	75.65 ± 12.37 a	42.07
70 CP	69.44 ± 8.86 a,b	39.09
100 CP	50.11 ± 9.00 c	18.95
30 NB	46.21 ± 11.06 c,d	8.46
70 NB	45.01 ± 11.27 c,d,e	3.81
100 NB	27.06 ± 7.60 f	- 11.76
2 ALB	36.7 ± 4.24 g	- 10.77
M-AIN-93G	48.03 ± 10.85 c,d,e,h	11.91

[1] $F = 29.07$ $p < 0.0001$ $R = 0.86$ $R^2 = 0.74$
[2] $F = 28.78$ $p < 0.0001$ $R = 0.89$ $R^2 = 0.79$

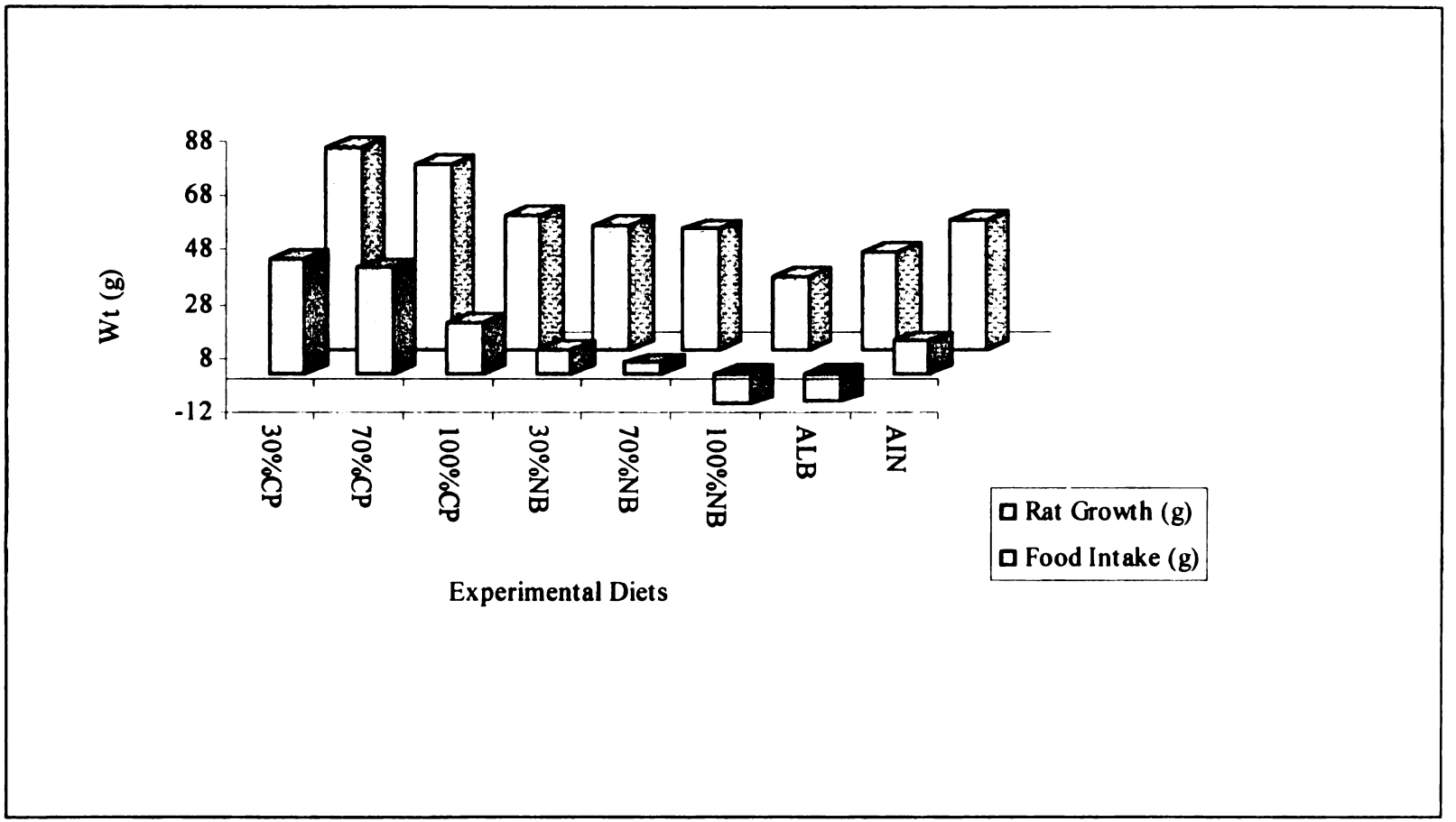

Figure 42 Average Food Intake of Experimental Diets and Rat Growth over the Feeding Period

The data on fecal matter for each diet is shown in Table 43, and indicates that fecal weight and fecal protein content was higher for all experimental diets compared to the modified AIN-93G diet. Fecal protein content was highest in the 100% diets, while fecal weight was highest in the 70% diets. The higher excretion of nitrogen is likely to originate in part from microbial growth in the large intestine and reflects lower protein digestibility of that diet. The effect of food intake, diet and their interaction on fecal protein content indicates that only food intake had a highly significant effect on fecal protein ($p < 0.0001$), $R = 0.81$ and $R^2 = 0.65$.

Table 43 Protein content and weight of fecal matter during feeding study

Diet	Protein content (% db)	Wt (g)
30CP	21.54 ± 2.24 a	8.76 ± 1.67 a
70 CP	22.99 ± 0.85 a,b	11.15 ± 2.12 b
100 CP	26.16 ± 1.49 b,c	8.89 ± 1.69 a,c
30 NB	20.33 ± 1.14 a,b,d	8.25 ± 0.87 a,c,d
70 NB	18.64 ± 2.51 a,d,e	10.48 ± 1.42 a,b,c,e
100 NB	24.12 ± 0.98 a,b,c,d,f	7.59 ± 2.85 a,c,d,f
2% ALB	10.40 ± 1.22 g	7.69 ± 1.25 a,c,d,f,g
Modified AIN-93G	10.78 ± 2.46 g,h	7.95 ± 2.02 a,c,d,f,g,h

[1] n = 3
[2] Means within a column followed by the same letters are not significantly different ($p < 0.0001$)

The average apparent (AD) and true digestibility (TD) are shown in Table 44 and indicates that TD ranges from 62.6% for 100%NB to 98.1% for the modified AIN-93G diet. The average values for CP diets ranged from 73.7 % - 87.5% and 62.6% - 78.2% for NB diets. All of the CP diets had higher digestibilities than the NB diets, and the 30%CP and 30%NB diets contained the highest TD from each of their groups. TD of 30%CP diet was not significantly different from the modified AIN-93G diet; all others were significantly different. The effect of diet and food intake on TD indicates that diet and the interaction of food intake*diet had a highly significant effect ($p < 0.0001$).

Table 44 Average *in-vivo* Apparent Protein Digestibility (AD) and True Protein Digestibility (TD) and Standard Deviation of the experimental Diets

Diets	AD	TD
30 CP	76.56 ± 5.03 a	87.49 ± 5.32 a
70 CP	61.42 ± 8.41 b	73.74 ± 8.79 b
100 CP	64.13 ± 8.53 c	74.61 ± 9.28 c
30 NB	49.48 ± 12.09 d	78.23 ± 9.30 d
70 NB	64.09 ± 8.57 e	69.39 ± 19.56 e
100 NB	17.21 ± 15.12 f	62.64 ± 21.61 f
Modified AIN-93G	75.65 ± 9.03 g	98.15 ± 9.41a,g

[1] n = 8

[2] Means within a column followed by the same letters are not significantly different ($p < 0.0001$)

Estimates of the protein nutritional quality (RPER, RNPR, RTD and PDCAAS) of the experimental diets are shown in Table 45. It indicates that the 30% diets generally had the highest protein quality while the 100% diets had the lowest quality. The 100%NB diet was the only diet that had zero protein quality using PER and NPR because this diet did not support rat growth. The PDCAAS values (based on human requirements) were highest for the 70% diets, values over 100% were observed for the 70%CP diet and modified AIN-93G. In the methods that assess protein quality based on growth, the RNPR values of experimental diets were higher than the RPER values because the RNPR method also credits protein used for both growth and maintenance.

Table 45 Estimates of Protein Nutritional Quality of Cowpea and Navy Bean Diets from Relative Protein Efficiency Ratio (RPER) [1], Relative Net Protein Ratio (RNPR) [2], Relative True Protein Digestibility (RTD)[3], and Protein Digestibility Corrected Amino Acid Score (PDCAAS)[4]

Diet	RPER	RNPR	RTD	PDCAAS *in-vivo*	PDCAAS pH-Stat	PDCAAS pH-Drop
30CP	138	339	89.14	89.24	84.19	81.22
70CP	144	329	75.13	95.12	106.48	99.81
100CP	81	113	76.01	58.20	62.79	60.70
30NB	103	121	79.70	74.32	75.32	71.15
70NB	73	39	70.69	74.25	86.57	71.44
100NB	0	0	63.82	38.84	49.15	40.00

[1] RPER = PER of test diet / PER of modified AIN-93G
[2] RNPR = NPR of test diet / NPR of modified AIN-93G
[3] RTD = TD of test protein / TD of modified AIN-93G
[4] PDCAAS of test protein from *in-vivo* and *in-vitro* assays

The growth tests, RPER and RNPR, showed high significant correlations with each other (r = 0.90). The scoring methods correlated highest with each other, *in-vivo* PDCAAS vs. pH-Stat PDCAAS, r = 0.94; *in-vivo* PDCAAS vs. pH-Drop PDCAAS, r = 0.97; and pH-Stat PDCAAS vs. pH-Drop PDCAAS, r = 0.97). The lowest correlations were found between the scoring methods and RTD, pH-Stat PDCAAS and RTD, r = 0.41; pH-Drop PDCAAS and RTD, r = 0.57; and *in-vivo* PDCAAS vs. RTD, r = 0.69. The growth tests showed medium to high correlations with the scoring tests.

Harris et al (1988) reported significant correlations (r = 0.90) between NPR and PER, and Pellett and Young (1981) and the Codex Alimentarius Commission (1984) reported that RNPR was the most appropriate rat test for routine assessment of protein quality of foods for humans. They found that NPR method gave similar results to net protein utilization (NPU) and biological value (BV).

Sarwar (1997) reported that PDCAAS overestimated the protein quality of protein sources that contained antinutritional factors, particularly when the scores were calculated based on human requirements. He found that mustard flour and black beans had a PDCAAS of 84% and 45% respectively compared to RPER and RNPR of 0. This was also observed for 100%NB diet in this study, with PDCAAS of 49%, 40% and 39% by pH-Stat, pH-Drop and *in-vivo* assays respectively, compared to 0 for RPER and RNPR. He suggested that vegetable protein sources contain growth-depressing factors such as isothiocyanates in mustard flour, trypsin inhibitors and lectins in black beans and lysinoalanine in alkaline-treated soy isolate. Sarwar (1997), Eggum et al (1989) and Sarwar and Peace (1986) reported that the PDCAAS method also does not take into account the bioavailability of individual amino acids, which may be up to 40% lower than

the overall digestibility of protein in the same food. They concluded that the PDCAAS method may give misleading results about the quality of proteins co-limiting in more than one essential amino acid.

Rubio et al (1998) reported on the nutritional utilization of chickpea and its isolated globulin proteins by rats. As expected, they found that growth of rats was impaired when chickpea meal (uncooked) was included as the sole source of protein in the diet, and was due to interference with systemic protein metabolism. They also added that the lower protein efficiency observed was not due to the presence of insoluble residue (starch and fiber) in the diet, since the presence of fiber in similar quantities in the control diet had no detrimental effect on performance of rats or the NPU values.

Supplementation of cooked bean diets with limiting amino acids has been extensively reported to improve protein quality. Kakade and Evans, (1965) found that autoclaved navy beans supplemented with either methionine alone or with all the limiting essential amino acids had a similar PER as casein. Bressani et al, (1963) reported an improved an improved PER and BV for black beans when supplemented with 0.2% methionine. Sarwar (1997) reported on the beneficial effects of supplementation with limiting amino acids. He suggested that the PDCAAS method assumes complete biological efficiency of the supplemented amino acids. In his study on amino acid supplemented zein, a protein of low digestibility and poor quality, he found a marked difference between the PDCAAS and RPER or RNPR of amino acid supplemented zein. Supplementation of cooked beans with other protein sources including corn, wheat, rice and oats, has also

been reported in the literature. Yadav and Liener (1977) reported similar PER values for navy bean supplemented with various cereals compared to the PER for casein.

There is much interest in determining the optimum nutritional ratio of consumption between the cereal and legume protein sources. Bressani and Elias (1974) have established this ratio as 2.6:1, which corresponds to a diet constituted by 72 parts corn and 28 parts bean. Arroyave (1973) reported that children fed a corn : bean diet, in a 70:30 ratio by weight were able to meet their protein and calorie needs. However, Murillo et al (1974) suggested that this ratio was too bulky for the weight and size of laboratory animals due to their gastric capacity. Bressani et al (1980) however found that a 70:30 corn : bean diet increases the consumption of the diet mixture in 5 week old pigs fed ad libitum. This supported the results of this study that the 30% legume diets had greater digestibilities compared to the other experimental diets.

Conclusions

Results of this study indicated that the amino acid content of cowpea and navy bean residues decreased after heat processing. The protein quality of cowpea and navy bean diets was significantly lower than the modified AIN-93G control diet. All cowpea diets had higher protein quality than the navy bean diets. Cowpea or navy bean as the sole source of protein in the diet did not provide the pattern of essential amino acid requirements for pre-school children. Supplementation with wheat flour improved the protein quality of both legume diets. Lysine was the limiting amino acid for 30%CP, 30%NB and 70%NB diets, while the sulfur-amino acids were limiting in 70%CP, 100%CP, and 100%NB diets.

H_o: there are no significant differences between the protein quality of cowpea and navy bean protein diets compared to a modified AIN-93G diet.

Reject the H_o as stated and conclude that diet composition significantly affected the protein quality.

Future Research

The goals for future study include the following:

1. To determine the bioavailability of the limiting amino acids lysine and methionine in each diet so that a more accurate prediction of protein quality could be determined.

2. Research on the proportion of the total sulfur amino acid requirement which can be met by cystine for protein sources that have low cystine levels

SUMMARY AND RECOMMENDATIONS

There is considerable interest for increasing utilization of legumes as a protein source, particularly in countries where protein-energy malnutrition persists. The legumes chosen for study in this dissertation are of economic importance in the United States and a number of developing countries in Africa. Cowpeas are one of the major legumes consumed in the developing countries of Africa, particularly in West Africa, and navy beans are a major crop in the state of Michigan. Ultrafiltration processing has been increasingly utilized in the food industry to separate macromolecules such as proteins without adverse effects to chemical structure. Most of the research has been focused on dairy proteins, and increasingly on soybean proteins. There has been very little published on ultrafiltration processing of legumes other than soybeans. This research attempted to provide information on ultrafiltration processing of cowpea and navy beans and compositional, functional and nutritional characterization of the proteins fractions.

The results indicated the feasibility and potential for a number of value-added products from ultrafiltration processing of legumes. Aqueous extraction produced a high protein aqueous extract (protein concentrate) which was used for further ultrafiltration processing to produce legume protein isolates. These isolates, although different from a commercial soy protein isolate, had favorable functional and nutritional properties and could be utilized as ingredients in the food industry.

The high protein solubility of the isolates would be useful properties in liquid applications. Additionally, the high water absorption capacity of cowpea protein isolate also makes this fraction acceptable for liquid products. The high foam capacity and

stability of the navy bean isolate makes it acceptable for whipped products such as cakes, desserts and ice cream. Oil absorption capacity and emulsion capacity of the isolates, particularly navy bean isolate, suggests that they can be utilized in simulated meat products, cakes and dressings. The critical protein required for gelation was highest for soy protein isolate, which results in the experimental isolates being more acceptable for protein gels such as meats, cakes and cheese products. Protein digestibility of the isolates, determined by *in-vitro* assays, improved after ultrafiltration processing. This was associated with reduced lectin activity and increased amino acid content of the isolates.

The residue that remained after aqueous extraction is generally considered a waste by-product of the ultrafiltration process, and is either fed to animals or used as a soil amendment in organic farming. However, the results reported indicated that the residue had up to 40% of the total protein remaining after aqueous alkali extraction, and therefore had potential as human food. When the legume residue was combined in diets supplemented with wheat flour, it was found that diets with legume as the primary source of protein did not meet the nutritional requirements for pre-school children. However, a 30% cowpea-wheat flour diet blend was found to meet their nutritional requirements.

The results of these studies indicated that ultrafiltration processing has the potential to improve the utilization of legumes due to the fractionation of high quality, high value components while enabling tremendous savings in waste of residual components. The additional advantages of low operation costs, and simplicity in operation, make ultrafiltration processing an important technology for increased utilization in the food industry. Typical annual operating costs including maintenance, replacement membranes and electricity are generally about 10% of the initial capital

investment. This is significantly lower than the average operating cost of other types of technologies currently in use by the food industry, which may range from 25 – 40% of the initial investment.

The success of ultrafiltration processing into emerging economies must be based on the existence of a demand for the processed product, a demand that can be satisfied profitably. Current statistics on global hunger and poverty, and protein malnutrition suggests that this processing technology could play a major role in improving global food security. This would be possible mainly through the production of safe and nutritious food for the consumer, as well as providing a source of livelihood, and therefore money to access food. The flexibility of ultrafiltration processing in terms of the production of a wide range of products ensures full usage of equipment. The choice of membrane systems can make it accessible to small, rural communities or large-scale commercial applications in urban areas. Due to the high initial capital cost, large-scale commercial operations would be recommended for technology transfer only to middle and high-income emerging economies.

The research conducted in this dissertation provides a framework for further research using other types of membrane modules such as mineral membranes, to assess the properties of the separated components, the reduction in fouling and hence improvement in yields. Additionally, an economic assessment is needed to evaluate if this technology is an "appropriate" technology for the developing world.

APPENDICES

APPENDIX 1

SUPPLEMENTARY MATERIAL

STUDY 1 OPTIMIZATION OF THE AQUEOUS EXTRACTION OF PROTEINS FROM COWPEAS (Vigna unguiculata) AND NAVY BEANS (Phaseolus vulgaris)

Table 46 Protein content, Predicted Protein content, Soluble Solids and Protein Yield from Cowpea Flour (CP) after extraction under various conditions of pH, particle size and temperature

Sample	pH	Particle size (mm)	Temperature (°C)	Protein in extract (%)	Predicted Protein (%)	Soluble Solids (%)	Protein Yield (g protein / 100 g flour)
CP	2	0.79	25	25.58	24.87	2.23	13.67
CP	4	0.79	25	16.63	21.67	2.71	10.29
CP	6	0.79	25	29.20	28.72	3.37	22.69
CP	8	0.79	25	33.70	32.39	2.91	22.60
CP	10	0.79	25	34.16	34.29	3.43	27.48
CP	12	0.79	25	35.76	35.85	4.89	40.63
CP	2	1.59	25	15.55	24.32	2.91	10.77
CP	4	1.59	25	11.18	21.11	2.81	7.09
CP	6	1.59	25	29.56	28.16	3.08	20.54
CP	8	1.59	25	35.68	31.84	3.03	24.32
CP	10	1.59	25	37.35	33.73	4.04	34.45
CP	12	1.59	25	38.00	35.30	5.07	35.75
CP	2	2.36	25	19.37	20.90	2.56	11.80
CP	4	2.36	25	17.68	17.69	2.71	11.57
CP	6	2.36	25	21.34	24.75	2.74	13.12
CP	8	2.36	25	31.92	28.42	2.66	19.33
CP	10	2.36	25	32.85	30.32	3.27	23.56
CP	12	2.36	25	34.44	31.88	4.69	34.63
CP	2	3.17	25	17.29	19.99	2.47	9.44
CP	4	3.17	25	16.17	16.78	2.42	8.73
CP	6	3.17	25	19.30	23.84	2.62	11.84
CP	8	3.17	25	30.90	27.51	2.40	16.47
CP	10	3.17	25	30.97	29.41	1.85	12.79
CP	12	3.17	25	31.01	30.97	3.78	24.22
CP	2	6.35	25	14.64	15.98	2.29	7.90
CP	4	6.35	25	11.04	12.77	2.01	4.74

Table 46 (cont'd)

Sample	pH	Particle size (mm)	Temperature (°C)	Protein in extract (%)	Predicted Protein (%)	Soluble Solids (%)	Protein Yield (g protein / 100 g flour)
CP	6	6.35	25	22.79	19.83	1.57	8.10
CP	8	6.35	25	23.03	23.50	3.35	17.04
CP	10	6.35	25	23.22	25.40	1.67	8.88
CP	12	6.35	25	26.63	26.96	3.30	21.12
CP	2	0.79	50	26.24	25.65	2.38	15.43
CP	4	0.79	50	20.70	22.45	3.08	16.14
CP	6	0.79	50	26.05	29.50	1.14	7.01
CP	8	0.79	50	36.08	33.17	2.99	23.55
CP	10	0.79	50	36.14	35.07	3.05	24.07
CP	12	0.79	50	37.40	36.63	4.65	36.61
CP	2	1.59	50	23.14	25.10	2.79	15.36
CP	4	1.59	50	18.32	21.89	1.68	7.15
CP	6	1.59	50	30.47	28.94	3.12	22.13
CP	8	1.59	50	31.63	32.62	3.48	24.15
CP	10	1.59	50	32.41	34.51	3.90	28.00
CP	12	1.59	50	34.76	36.08	4.65	38.16
CP	2	2.36	50	17.10	21.68	4.86	19.20
CP	4	2.36	50	14.76	18.47	2.54	8.68
CP	6	2.36	50	29.98	25.53	2.93	19.64
CP	8	2.36	50	30.84	29.20	0.75	4.90
CP	10	2.36	50	31.05	31.10	3.99	24.41
CP	12	2.36	50	35.16	32.66	4.32	34.87
CP	2	3.17	50	22.45	20.77	2.63	13.38
CP	4	3.17	50	21.61	17.57	2.61	12.13
CP	6	3.17	50	25.87	24.62	2.50	13.05
CP	8	3.17	50	29.13	28.29	2.82	19.14

Table 46 (cont'd)

Sample	pH	Particle size (mm)	Temperature (°C)	Protein in extract (%)	Predicted Protein (%)	Soluble Solids (%)	Protein Yield (g protein / 100 g flour)
CP	10	3.17	50	30.67	30.19	2.27	16.78
CP	12	3.17	50	31.02	31.75	4.81	32.03
CP	2	6.35	50	17.83	16.76	2.50	9.90
CP	4	6.35	50	13.24	13.56	2.13	6.27
CP	6	6.35	50	23.55	20.61	1.59	8.05
CP	8	6.35	50	28.15	24.28	1.51	9.56
CP	10	6.35	50	29.31	26.18	1.76	11.75
CP	12	6.35	50	29.77	27.74	2.78	18.14

Table 47 Protein content, Predicted Protein content, Soluble Solids and Protein Yield from Navy bean (NB) Flour after extraction under various conditions of pH, particle size and temperature

Sample	pH	Particle size (mm)	Temperature (°C)	Protein in extract (%)	Predicted Protein (%)	Soluble Solids (%)	Protein Yield (g protein / 100 g flour)
NB	2	0.79	25	24.54	26.23	2.38	6.84
NB	4	0.79	25	25.16	23.02	3.08	10.53
NB	6	0.79	25	32.55	30.07	1.14	28.11
NB	8	0.79	25	33.11	33.75	2.99	25.86
NB	10	0.79	25	37.61	35.64	3.05	16.97
NB	12	0.79	25	40.73	37.21	4.65	48.96
NB	2	1.59	25	28.43	25.67	2.79	6.91
NB	4	1.59	25	27.34	22.46	1.68	24.20
NB	6	1.59	25	32.19	29.52	3.12	37.41
NB	8	1.59	25	29.68	33.19	3.48	35.88
NB	10	1.59	25	35.14	35.09	3.90	19.18
NB	12	1.59	25	38.52	36.65	4.65	55.06
NB	2	2.36	25	24.51	22.25	4.86	7.01
NB	4	2.36	25	22.08	19.05	2.54	10.05
NB	6	2.36	25	23.58	26.10	2.93	14.49
NB	8	2.36	25	27.03	29.77	0.75	19.03
NB	10	2.36	25	33.56	31.67	3.99	21.20
NB	12	2.36	25	35.60	33.23	4.32	32.41
NB	2	3.17	25	27.28	21.35	2.63	3.79
NB	4	3.17	25	25.73	18.14	2.61	17.44
NB	6	3.17	25	25.20	25.19	3.91	22.46
NB	8	3.17	25	22.02	28.87	3.13	15.78
NB	10	3.17	25	28.32	30.76	1.85	15.35
NB	12	3.17	25	29.38	32.33	3.78	20.88
NB	2	6.35	25	21.54	17.34	2.29	2.88
NB	4	6.35	25	11.14	14.13	2.01	1.25

Table 47 (cont'd)

Sample	pH	Particle size (mm)	Temperature (°C)	Protein in extract (%)	Predicted Protein (%)	Soluble Solids (%)	Protein Yield (g protein / 100 g flour)
NB	6	3.17	25	25.20	25.19	3.91	22.46
NB	8	3.17	25	22.02	28.87	3.13	15.78
NB	10	3.17	25	28.32	30.76	1.85	15.35
NB	12	3.17	25	29.38	32.33	3.78	20.88
NB	2	6.35	25	21.54	17.34	2.29	2.88
NB	4	6.35	25	11.14	14.13	2.01	1.25
NB	6	6.35	25	17.22	21.18	1.57	4.42
NB	8	6.35	25	21.97	24.86	3.35	4.40
NB	10	6.35	25	25.47	26.75	1.67	0.68
NB	12	6.35	25	25.42	28.32	3.30	4.85
NB	2	0.79	50	26.24	27.01	2.38	18.72
NB	4	0.79	50	24.27	23.08	3.08	15.22
NB	6	0.79	50	30.83	30.85	1.14	27.23
NB	8	0.79	50	32.42	34.53	2.99	21.00
NB	10	0.79	50	32.44	36.43	3.05	29.87
NB	12	0.79	50	39.27	37.99	4.65	40.82
NB	2	1.59	50	25.89	26.45	2.79	15.58
NB	4	1.59	50	23.49	23.24	1.68	13.41
NB	6	1.59	50	32.17	30.30	3.12	27.18
NB	8	1.59	50	35.73	33.97	3.48	18.39
NB	10	1.59	50	38.35	35.87	3.90	39.08
NB	12	1.59	50	38.48	37.43	4.65	36.00
NB	2	2.36	50	24.51	23.03	1.12	12.42
NB	4	2.36	50	22.08	19.83	1.91	12.95
NB	6	2.36	50	23.58	26.88	2.53	15.66
NB	8	2.36	50	27.03	30.56	3.09	21.75

Table 47 (cont'd)

Sample	pH	Particle size (mm)	Temperature (°C)	Protein in extract (%)	Predicted Protein (%)	Soluble Solids (%)	Protein Yield (g protein / 100 g flour)
NB	10	2.36	50	33.56	32.45	2.72	23.95
NB	12	2.36	50	35.60	34.02	4.27	28.83
NB	2	3.17	50	27.28	22.13	0.54	8.27
NB	4	3.17	50	25.73	18.92	3.16	10.50
NB	6	3.17	50	25.20	25.97	3.91	6.14
NB	8	3.17	50	22.02	29.65	3.13	17.58
NB	10	3.17	50	28.32	31.54	2.31	20.21
NB	12	3.17	50	29.38	33.11	3.68	22.17
NB	2	6.35	50	21.54	18.12	0.53	8.51
NB	4	6.35	50	11.14	14.91	0.50	8.62
NB	6	6.35	50	17.22	21.96	1.04	11.52
NB	8	6.35	50	21.97	25.64	0.85	8.82
NB	10	6.35	50	25.47	27.53	0.16	10.68
NB	12	6.35	50	25.42	29.10	1.08	12.59

Table 48 Contribution of pH, particle size, extraction temperature and bean type to protein content in cowpea and navy bean extract

Source	Nparm	DF	Sum of Squares	F Ratio	Prob. > F
pH	5	5	3099.87	62.76	< 0.0001
Particle size	4	4	1247.51	31.57	< 0.0001
Temperature	1	1	18.27	1.85	0.18
Bean	1	1	55.03	5.57	0.02

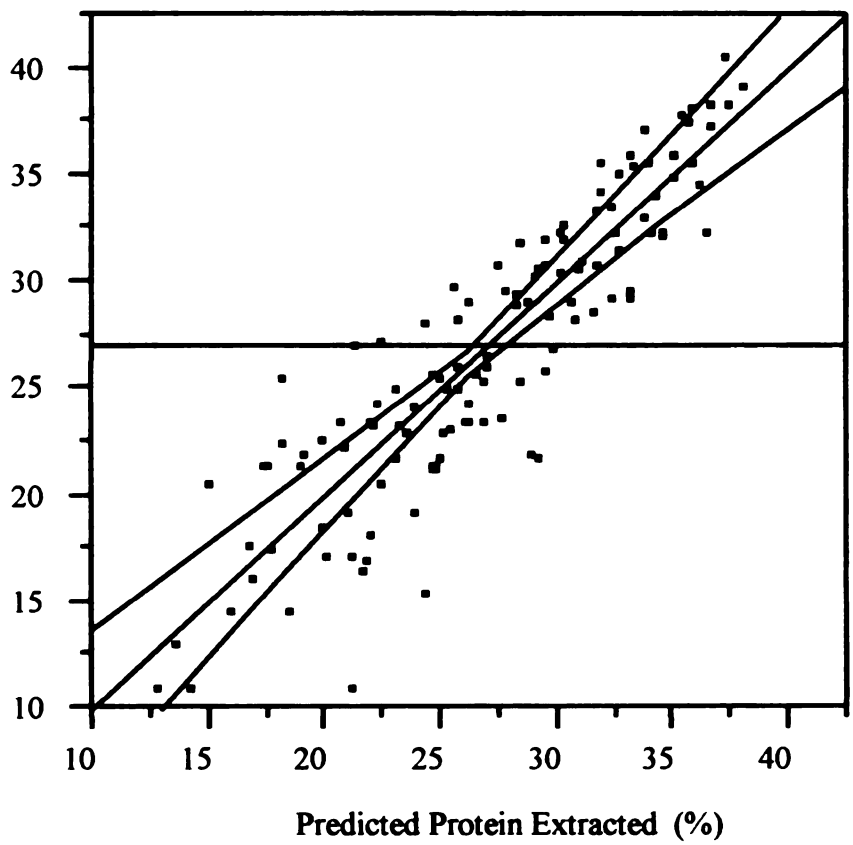

Figure 23 Leverage Plot for the Whole-Model Test of pH, temperature, particle size and bean type effects on % protein extracted from Cowpea and Navy Bean flour

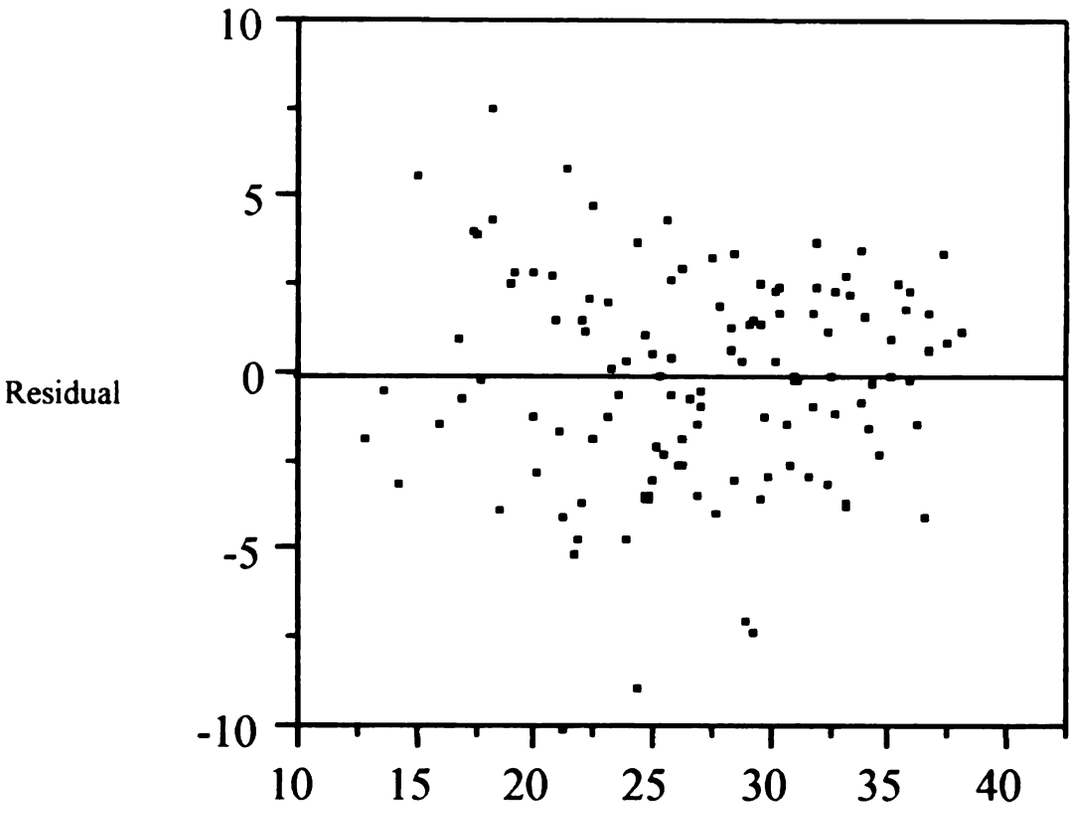

Figure 24 Residual Plot for the Whole-Model Test of pH, temperature, particle size and bean type effects on % protein extracted from Cowpea and Navy Bean flour

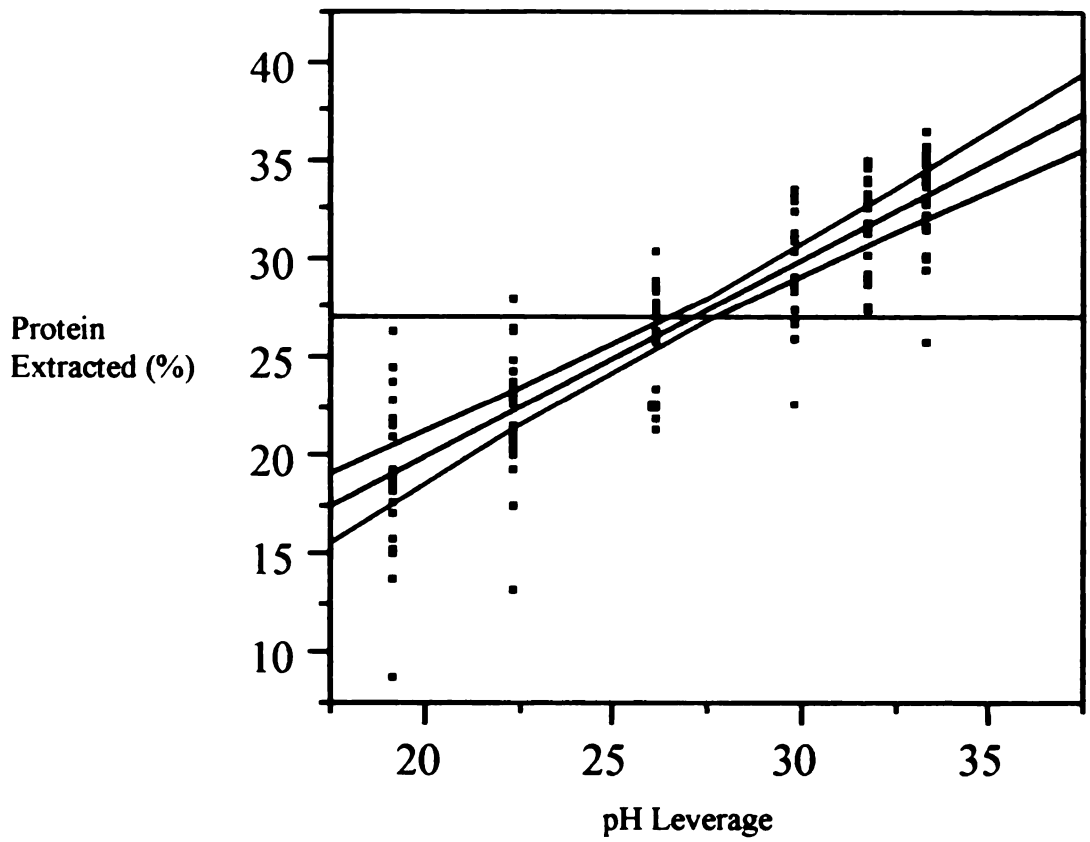

Figure 25 Leverage Plot for the significant effect pH on % protein extracted from Cowpea and Navy Bean flour

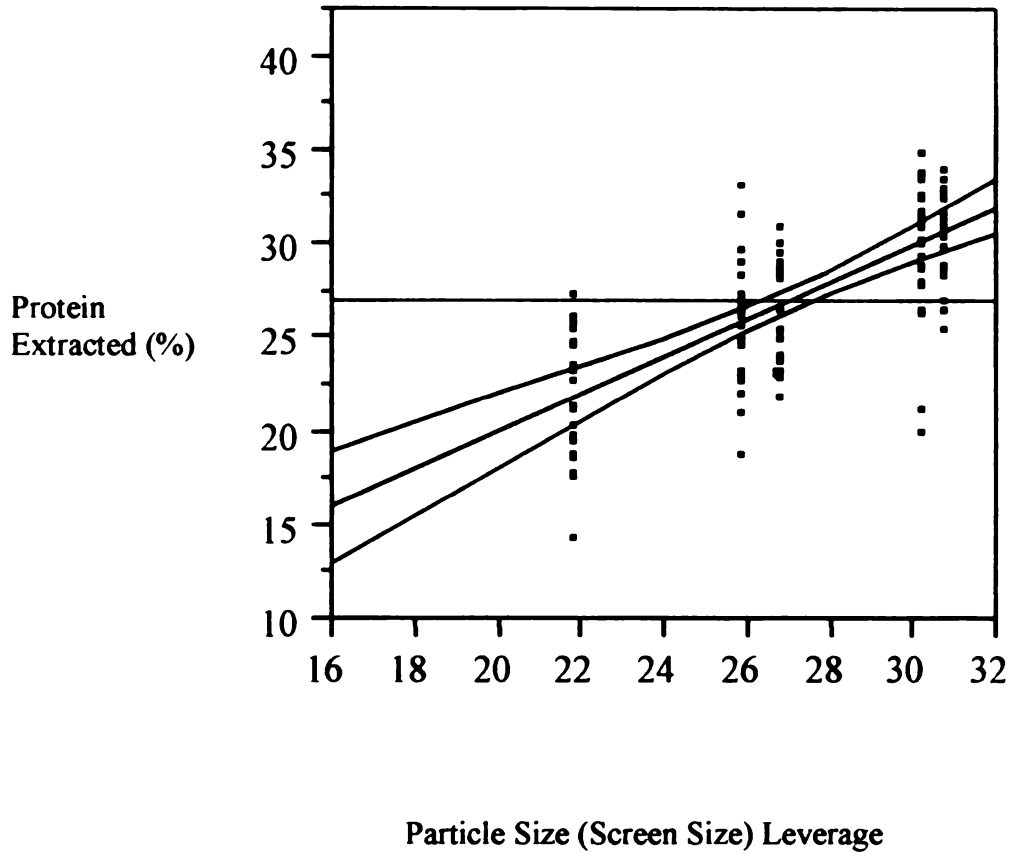

Figure 26 Leverage Plot for the significant effect particle size on % protein extracted from Cowpea and Navy Bean flour

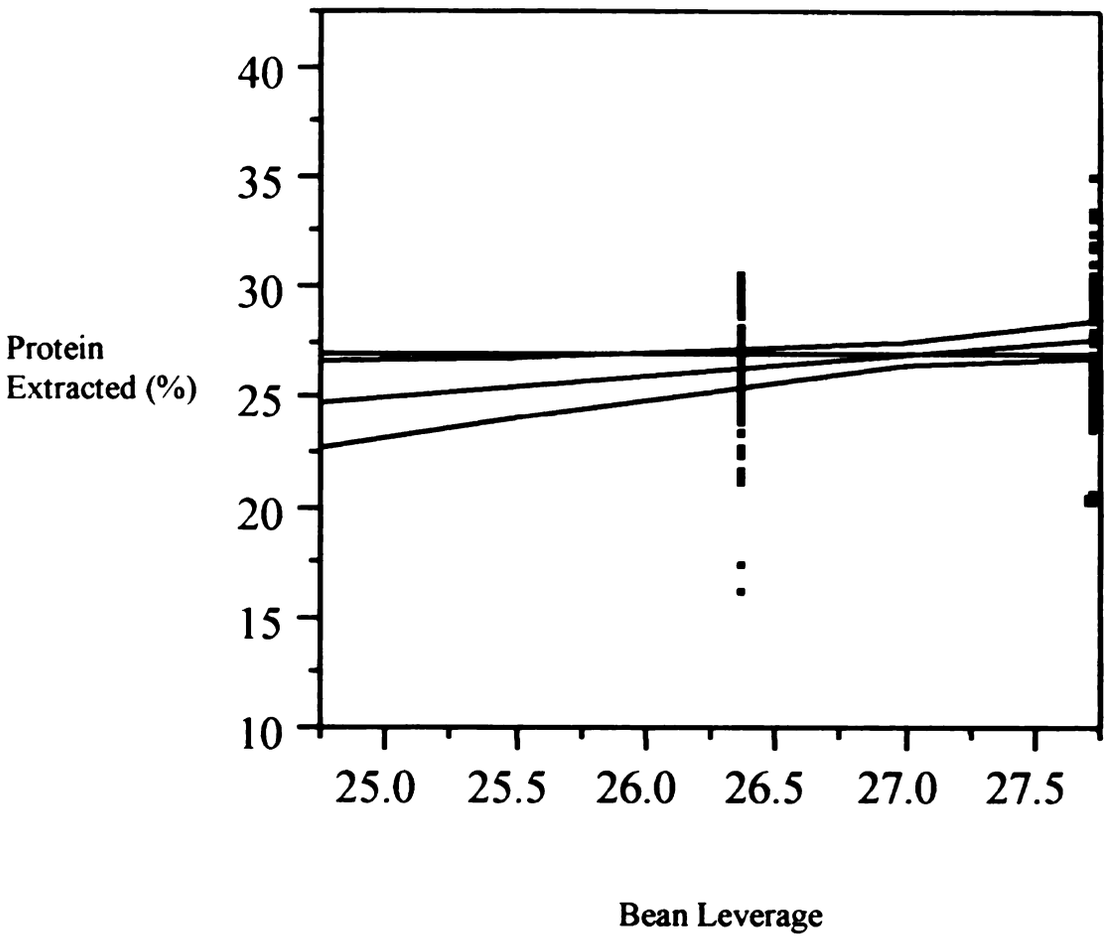

Figure 27 Leverage Plot for the significant effect bean type on % protein extracted from Cowpea and Navy Bean flour

APPENDIX 2

SUPPLEMENTARY MATERIAL

STUDY 2 ULTRAFILTRATION PROCESSING, CHARACTERIZATION AND FUNCTIONAL PROPERTIES OF COWPEA (*Vigna unguiculata*) AND NAVY BEAN (*Phaseolus vulgaris*) PROTEIN FRACTIONS

Table 49 Permeate and Retentate Flux during Ultrafiltration Processing of Aqueous Alkali Cowpea Extracts

Time (hr)	Flux (LMH)			
	Permeate 1	Permeate 2	Permeate 3	Retentate
0	0	0	0	0.00
0.02	8.34	9.04	1.39	3.48
0.03	7.30	6.95	1.74	1.91
0.07	8.69	6.08	1.74	1.74
0.10	8.23	6.37	1.27	3.71
0.17	6.26	3.96	0.83	3.48
0.20	5.50	4.00	0.71	2.90
0.23	4.97	3.58	0.62	3.38
0.27	4.56	3.39	0.55	3.13
0.30	4.25	3.21	0.50	3.05
0.33	4.17	3.06	0.46	2.85
0.37	4.11	2.80	0.42	2.84
0.42	3.73	2.59	0.39	2.73
0.50	3.48	2.36	0.35	2.64
0.58	3.30	2.20	0.31	2.46
0.67	3.16	2.05	0.29	2.40
0.83	2.96	1.85	0.25	2.06
1.08	2.72	1.59	0.20	1.63
1.33	2.55	1.37	0.19	1.33
1.67	2.50	1.27	0.16	1.30
2.00	2.56	1.35	0.17	1.16
3.00	2.74	1.58	0.12	1.45
4.00	2.80	2.03	0.18	1.46
5.00	2.77	2.06	0.20	1.48
6.00	2.51	2.01	0.21	1.64
6.75	2.57	2.06	0.23	2.35

Table 50 Permeate and Retentate Flux during Ultrafiltration Processing of Aqueous Alkali Navy Bean Extracts

Time (hr)	Flux (LMH)			
	Permeate 1	Permeate 2	Permeate 3	Retentate
0.00	0.00	0.00	0.00	0.00
0.02	6.95	7.65	7.79	15.64
0.03	6.26	5.56	5.91	20.86
0.07	6.60	4.34	4.69	18.25
0.10	5.91	3.71	4.87	15.64
0.17	5.98	3.27	4.03	13.90
0.20	5.49	3.19	3.88	13.32
0.23	4.92	2.93	3.82	12.91
0.27	4.65	2.91	3.74	12.60
0.30	4.44	2.90	3.78	12.94
0.33	4.14	2.68	3.65	12.51
0.37	3.95	2.97	3.79	12.64
0.42	3.61	2.78	3.67	12.23
0.50	3.50	2.78	3.48	11.59
0.58	3.10	2.70	3.58	11.72
1.08	2.78	1.60	2.21	7.22
1.67	2.64	1.31	2.13	5.56
3.33	2.50	1.22	1.34	3.65
5.00	1.93	1.07	0.94	2.53
5.83	2.09	1.14	0.85	2.19

Table 51 Volume of Aqueous Alkali Cowpea Extracts during Ultrafiltration Processing

Time (hr)	Volume (L)			
	Permeate 1	Permeate 2	Permeate 3	Retentate
0	0.00	0.00	0.00	0.00
0.02	0.12	0.13	0.02	0.05
0.03	0.21	0.20	0.05	0.06
0.07	0.50	0.35	0.10	0.10
0.10	0.71	0.55	0.11	0.32
0.17	0.90	0.57	0.12	0.50
0.20	0.95	0.69	0.12	0.50
0.23	1.00	0.72	0.13	0.68
0.27	1.05	0.78	0.13	0.72
0.30	1.10	0.83	0.13	0.79
0.33	1.20	0.88	0.13	0.82
0.37	1.30	0.89	0.13	0.90
0.42	1.34	0.93	0.14	0.98
0.50	1.50	1.02	0.15	1.14
0.58	1.66	1.11	0.16	1.24
0.67	1.82	1.18	0.16	1.38
0.83	2.13	1.33	0.18	1.48
1.08	2.54	1.49	0.19	1.52
1.33	2.94	1.58	0.22	1.53
1.67	3.60	1.83	0.23	1.87
2.00	4.42	2.33	0.30	2.00
3.00	7.10	4.10	0.32	3.75
4.00	9.65	7.00	0.63	5.05
5.00	11.95	8.90	0.85	6.40
6.00	13.00	10.42	1.11	8.50
6.75	15.00	12.00	1.32	13.70

Table 52 Flow rate of Aqueous Alkali Cowpea Extracts during Ultrafiltration Processing

Time (hr)	Flow rate (L/hr)			
	Permeate 1	Permeate 2	Permeate 3	Retentate
0	0	0	0	0
0.02	7.20	7.80	1.20	3.00
0.03	6.30	6.00	1.50	1.65
0.07	7.50	5.25	1.50	1.50
0.10	7.10	5.50	1.10	3.20
0.17	5.40	3.42	0.72	3.00
0.20	4.75	3.45	0.61	2.50
0.23	4.29	3.09	0.54	2.91
0.27	3.94	2.93	0.48	2.70
0.30	3.67	2.77	0.43	2.63
0.33	3.60	2.64	0.40	2.46
0.37	3.55	2.41	0.37	2.45
0.42	3.22	2.23	0.34	2.35
0.50	3.00	2.04	0.30	2.28
0.58	2.85	1.90	0.27	2.13
0.67	2.73	1.77	0.25	2.07
0.83	2.56	1.60	0.22	1.78
1.08	2.34	1.38	0.18	1.40
1.33	2.21	1.19	0.17	1.14
1.67	2.16	1.10	0.14	1.12
2.00	2.21	1.17	0.15	1.00
3.00	2.37	1.37	0.11	1.25
4.00	2.41	1.75	0.16	1.26
5.00	2.39	1.78	0.17	1.28
6.00	2.17	1.74	0.19	1.42
6.75	2.22	1.78	0.20	2.03

Table 53 Volume of Aqueous Alkali Navy Bean Extracts during Ultrafiltration Processing

Time (hr)	Volume (L)			
	Permeate 1	Permeate 2	Permeate 3	Retentate
0.00	0.00	0.00	0.00	0.00
0.02	0.10	0.11	0.11	0.23
0.03	0.18	0.16	0.17	0.60
0.07	0.38	0.25	0.27	1.05
0.10	0.51	0.32	0.42	1.35
0.17	0.86	0.47	0.58	2.00
0.20	0.95	0.55	0.67	2.30
0.23	0.99	0.59	0.77	2.60
0.27	1.07	0.67	0.86	2.90
0.30	1.15	0.75	0.98	3.35
0.33	1.19	0.77	1.05	3.60
0.37	1.25	0.94	1.20	4.00
0.42	1.30	1.00	1.32	4.40
0.50	1.51	1.20	1.50	5.00
0.58	1.56	1.36	1.80	5.90
1.08	2.60	1.50	2.07	6.75
1.67	3.80	1.88	3.07	8.00
3.33	7.20	3.50	3.85	10.50
5.00	8.35	4.61	4.07	10.90
5.83	10.50	5.72	4.27	11.01

Table 54 Flow rate (L/hr) of Aqueous Alkali Navy Bean Extracts during Ultrafiltration Processing

Time (hr)	Flow rate (L/hr)			
	Permeate 1	Permeate 2	Permeate 3	Retentate
0.00	0.00	0.00	0.00	0.00
0.02	6.00	6.60	6.72	13.50
0.03	5.40	4.80	5.10	18.00
0.07	5.70	3.75	4.05	15.75
0.10	5.10	3.20	4.20	13.50
0.17	5.16	2.82	3.48	12.00
0.20	4.74	2.75	3.35	11.50
0.23	4.24	2.53	3.30	11.14
0.27	4.01	2.51	3.23	10.88
0.30	3.83	2.50	3.27	11.17
0.33	3.57	2.31	3.15	10.80
0.37	3.41	2.56	3.27	10.91
0.42	3.12	2.40	3.17	10.56
0.50	3.02	2.40	3.00	10.00
0.58	2.67	2.33	3.09	10.11
1.08	2.40	1.38	1.91	6.23
1.67	2.28	1.13	1.84	4.80
3.33	2.16	1.05	1.16	3.15
5.00	1.67	0.92	0.81	2.18
5.83	1.80	0.98	0.73	1.89

Table 55 Protein Recovered (% of Aqueous Extract) after Extraction at pH 10 and 25°C

Samples	Protein Recovered (% of Extract)	
	Cowpea	*Navy Bean*
Isolate	75.6	72.6
Permeate 1	7.49	4.70
Permeate 2	3.63	2.14
Permeate 3	0.67	1.98
Total	***87.48***	***81.43***

Table 56 Retention Times and Area under the peak (μVolt-sec) of Amino Acids of Cowpea and Navy Bean Fractions

Amino Acid	RET TIME	CPF	CPPE	CPI	NBF	NBPE	NBI	SPI	CASEIN
Asp	4.39	441.09	605.49	708.84	569.47	997.45	543.20	714.36	1288.78
Glu	4.90	708.30	935.52	1121.13	746.15	1709.11	704.16	1082.11	3421.85
Ser	7.93	382.10	388.52	474.05	523.60	686.56	143.66	345.28	1461.92
Gly	8.70	650.76	687.49	776.61	705.54	822.89	209.23	545.54	1227.45
His	10.09	390.20	422.84	470.75	356.56	150.95	137.18	351.03	618.91
Arg	11.89	262.07	283.51	339.33	359.27	955.87	421.89	179.54	963.06
Thr	12.64	416.50	439.17	520.91	496.48	772.33	374.74	299.08	975.13
Ala	13.19	102.74	34.74	27.87	49.79	467.44	220.80	ND	105.99
Pro	13.92	410.99	391.00	470.82	395.58	ND	ND	457.08	3150.59
Tyr	21.22	134.55	148.13	198.41	153.04	105.15	145.04	188.79	956.23
Val	23.07	362.88	468.44	575.39	497.80	748.29	341.88	409.41	1644.74
Met	24.11	62.90	36.24	38.31	58.30	133.55	46.03	ND	445.22
Ile	27.96	311.35	375.04	455.03	404.17	517.96	247.60	245.40	722.91
Leu	28.60	531.77	566.27	701.07	666.89	907.48	431.47	413.89	370.71
Phe	ND	310.61	347.43	422.07	369.68	525.22	244.21	1029.38	978.09
Lys	36.15	739.40	815.79	993.68	875.69	1149.83	533.77	739.40	3054.57

ND – not dectectable

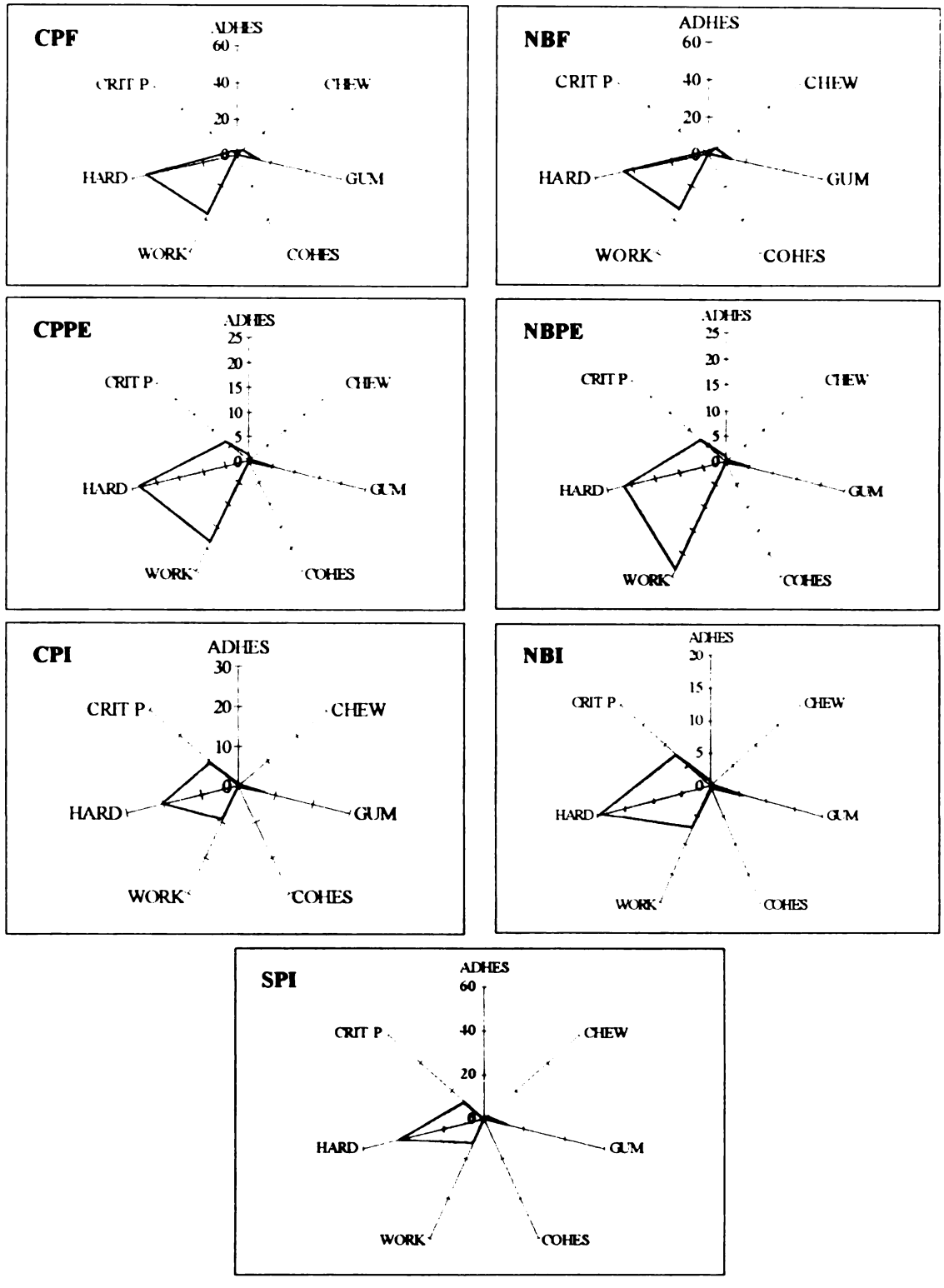

Figure 28 Texture Profile Analysis of Cowpea and Navy Bean Protein Gels (adhesiveness (ADHES), chewiness (CHEW), gumminess (GUM), cohesiveness (COHES), work done on gel (WORK), hardness (HARD), critical protein for gelation (CRIT P)

Table 57 Hunter Color Characteristics of Cowpea and Navy Bean Protein Fractions after Ultrafiltration Processing

Fractions	L	a	b
CPF	35.5	1.8	-14.5
CPE1	31.9	0.9	-10.4
CPE2	21.2	-1	-5.1
CPE3	19	0.2	-7
CPPE	23.4	0.6	-10.4
CPP1	18.7	0.9	-6.8
CPP2	19.1	0.4	-6.5
CPP3	18.6	0.7	-6.6
CPI	26.9	0.4	-3.4
NBF	35.9	2.2	-17.2
NBE1	16.9	1.6	-9.8
NBE2	17.5	0.7	-8.8
NBE3	14.5	1	-7.3
NBPE	16.7	1	-8.8
NBP1	12.1	-0.5	-3.1
NBP2	13.1	-0.3	-4
NBP3	12.5	-0.5	-3.4
NBI	15.6	0.9	-8.2
SPI	37.9	3.5	-23.1

Table 58 F-Values and Statistical Significance of Functional Properties of Cowpea and Navy Bean Protein Fractions after UF Processing

Variable	F-Value	P-Value
Hydration		
WAC	7401.30	< 0.0001
OAC	4681.69	< 0.0001
Protein Solubility	1121.72	< 0.0001
Structural/Rheological		
Critical Protein for Gelation	1710.42	< 0.0001
Work done on Gel	266.58	< 0.0001
Hardness of Gel	352.38	< 0.0001
Adhesiveness	319.76	< 0.0001
Chewiness	846.42	< 0.0001
Gumminess	318.98	< 0.0001
Cohesiveness	40.21	< 0.0001
T_p	50.56	< 0.0001
T_i	32.38	< 0.0001
ΔH	22.86	0.0003
Surface		
Foam Capacity	540.76	< 0.0001
Emulsion Capacity	3311.02	< 0.0001
Color		
L-Value	2648.50	< 0.0001
a-Value	155.88	< 0.0001
b-Value	1030.45	< 0.0001

Table 59 Correlation Matrix of Functional Properties of Cowpea and Navy Bean Protein Fractions [1]

	FC	*EC*	*WAC*	*OAC*	*PS*	*GEL P*	*WORK*	*HARD*	*ADHES.*	*CHEW.*	*GUM.*	*COHES.*	*%P*	*L*	*A*	*B*
FC	-															
EC	-0.05	-														
WAC	0.44	0.10	-													
OAC	-0.47	0.85	0.06	-												
PS	-0.13	-0.14	0.12	0.16	-											
Gel P	0.62	-0.74	0.36	-0.87	-0.01	-										
Work	-0.56	0.51	-0.30	0.61	-0.34	-0.83	-									
Hard	-0.42	0.02	-0.09	0.11	-0.28	-0.38	0.69	-								
Adhes.	-0.72	0.37	-0.69	0.59	0.09	-0.86	0.75	0.30	-							
Chew.	-0.52	0.30	0.02	0.42	-0.16	-0.59	0.76	0.93	0.39	-						
Gum.	-0.42	0.01	0.11	0.14	-0.11	-0.31	0.55	0.95	0.16	0.94	-					
Cohes.	0.46	-0.20	0.37	-0.17	0.78	0.41	-0.68	-0.58	-0.37	-0.54	-0.44	-				
%P	0.57	-0.73	0.39	-0.88	-0.13	0.94	-0.68	-0.07	-0.87	-0.30	0.00	0.25	-			
L	-0.49	-0.21	0.13	-0.01	-0.21	-0.12	0.43	0.88	0.04	0.82	0.93	-0.51	0.16	-		
a	0.15	-0.07	0.22	-0.27	-0.60	0.12	0.28	0.75	-0.30	0.61	0.72	-0.49	0.43	0.71	-	
b	0.02	0.14	0.04	0.31	0.65	-0.06	-0.32	-0.78	0.16	-0.62	-0.72	0.64	-0.37	-0.73	-0.95	-

[1] Absolute values of correlation coefficients ≥ 0.50 were significant at 5% levels. Absolute values ≥ 0.50 are underlined.

Table 60 Correlation Matrix of the Functional Properties of Cowpea Protein Fractions after Ultrafiltration Processing [1]

	FC	*EC*	*WAC*	*OAC*	*PS*	*Gel P*	*Work*	*Hard*	*Adhes.*	*Chew.*	*Gum.*	*Cohes.*	*%P*	*L*	*a*	*b*
FC	1.00															
EC	-0.90	1.00														
WAC	0.97	-0.79	1.00													
OAC	0.15	0.24	0.37	1.00												
PS	0.83	-0.53	0.94	0.67	1.00											
Gel P	0.86	-0.98	0.72	-0.37	0.44	1.00										
Work	-0.77	0.96	-0.62	0.49	-0.31	-0.99	1.00									
Hard	-0.59	0.86	-0.40	0.69	-0.05	-0.92	0.96	1.00								
Adhes.	-0.93	0.99	-0.83	0.20	-0.59	-0.98	0.95	0.83	1.00							
Chew.	-0.50	0.79	-0.28	0.78	0.07	-0.87	0.93	0.99	0.77	1.00						
Gum.	-0.35	0.70	-0.13	0.84	0.22	-0.78	0.86	0.95	0.66	0.98	1.00					
Cohes.	0.95	-0.76	0.97	0.37	0.92	0.69	-0.60	-0.38	-0.80	-0.27	-0.12	1.00				
%P	0.95	-0.98	0.85	-0.16	0.62	0.98	-0.93	-0.81	-1.00	-0.74	-0.62	0.83	1.00			
L	-0.30	0.65	-0.07	0.89	0.28	-0.74	0.82	0.94	0.61	0.97	0.99	-0.05	-0.58	1.00		
a	-0.65	0.88	-0.46	0.65	-0.12	-0.94	0.97	0.98	0.87	0.97	0.92	-0.44	-0.85	0.91	1.00	
b	0.95	-0.99	0.85	-0.17	0.61	0.98	-0.94	-0.81	-1.00	-0.75	-0.63	0.82	1.00	-0.58	-0.85	1.00

[1] Absolute values of correlation coefficients ≥ 0.50 were significant at 5% levels. Absolute values ≥ 0.50 are underlined.

Table 61 Correlation Matrix of the Functional Properties of Navy Bean Protein Fractions after Ultrafiltration Processing [1]

	FC	*EC*	*WAC*	*OAC*	*PS*	*Gel P*	*Work*	*Hard*	*Adhes.*	*Chew.*	*Gum.*	*Cohes.*	*%P*	*L*	*a*	*B*
FC	1.00															
EC	-0.97	1.00														
WAC	-0.97	1.00	1.00													
OAC	-0.99	1.00	0.99	1.00												
PS	0.37	-0.56	-0.57	-0.50	1.00											
Gel P	0.99	-0.99	-0.99	-1.00	0.48	1.00										
Work	-0.79	0.90	0.90	0.86	-0.85	-0.85	1.00									
Hard	-1.00	0.98	0.98	0.99	-0.41	-1.00	0.81	1.00								
Adhes.	-0.77	0.87	0.87	0.84	-0.82	-0.84	0.96	0.80	1.00							
Chew.	-0.99	0.96	0.95	0.98	-0.32	-0.98	0.75	0.99	0.73	1.00						
Gum.	-0.98	0.91	0.90	0.93	-0.17	-0.94	0.64	0.97	0.63	0.99	1.00					
Cohes.	0.76	-0.87	-0.89	-0.85	0.84	0.84	-0.95	-0.79	-0.89	-0.74	-0.63	1.00				
%P	0.91	-0.98	-0.98	-0.97	0.71	0.96	-0.96	-0.93	-0.93	-0.89	-0.81	0.95	1.00			
L	-1.00	0.98	0.97	0.99	-0.37	-0.99	0.79	1.00	0.77	1.00	0.98	-0.77	-0.92	1.00		
a	-0.96	0.97	0.97	0.96	-0.50	-0.96	0.87	0.96	0.84	0.94	0.90	-0.80	-0.94	0.95	1.00	
b	0.99	-0.98	-0.98	-0.99	0.41	1.00	-0.81	-1.00	-0.79	-0.99	-0.96	0.80	0.93	-1.00	-0.96	1.00

[1] Absolute values of correlation coefficients ≥ 0.50 were significant at 5% levels. Absolute values ≥ 0.50 are underlined.

APPENDIX 3

SUPPLEMENTARY MATERIAL

STUDY 3 NUTRITIONAL QUALITY OF COWPEA AND NAVY BEAN PROTEIN DIETS BY *IN VITRO* AND *IN VIVO* PROTEIN DIGESTIBILITY CORRECTED AMINO ACID SCORE (PDCAAS)

Table 62 Peak Area and Retention Time of Amino Acids of Cowpea and Navy Bean Diets

Amino Acid	Ret Time	30CP	70CP	100CP	30NB	70NB	100NB	M-AIN-93G
Asp	4.39	516.46	647.22	858.15	414.30	321.68	396.03	409.24
Glu	4.90	1606.78	1550.82	1406.37	1300.98	851.19	666.59	1317.39
Ser	7.93	550.25	681.12	689.26	434.12	474.92	468.15	584.21
Gly	8.70	956.69	1258.96	869.71	711.49	545.29	785.11	482.81
His	10.09	415.24	564.11	250.97	284.91	103.82	268.55	241.77
Arg	11.89	330.11	451.21	584.49	262.30	258.09	321.88	382.06
Thr	12.64	619.39	837.90	489.52	484.60	298.29	539.36	426.86
Ala	13.19	73.97	120.63	877.37	187.60	485.30	67.97	43.54
Pro	13.92	1089.09	981.40	653.25	837.49	572.57	438.14	1299.53
Tyr	21.22	190.83	240.57	242.04	138.34	132.81	127.12	270.10
Val	23.08	541.68	736.27	870.18	453.60	426.92	462.49	694.57
Met	24.11	258.08	139.57	105.82	71.66	145.32	61.88	178.52
Cys[1]	26.72	107.40	38.01	39.04	157.59	52.02	51.34	382.41
Ile	27.966	441.42	593.17	666.06	358.91	340.11	380.72	534.49
Leu	28.60	763.63	1014.80	1073.69	590.69	574.75	617.25	910.80
Phe	31.81	424.45	572.40	613.17	315.71	307.67	337.85	389.46
Trp[2]	34.10	327.94	198.08	149.45	251.44	215.07	141.44	676.28
Lys	36.15	766.95	1204.33	1463.05	523.55	651.47	806.12	1193.24

[1] Cys analysis by formic oxidation and acid hydrolysis
[2] Trp analysis by MSA hydrolysis

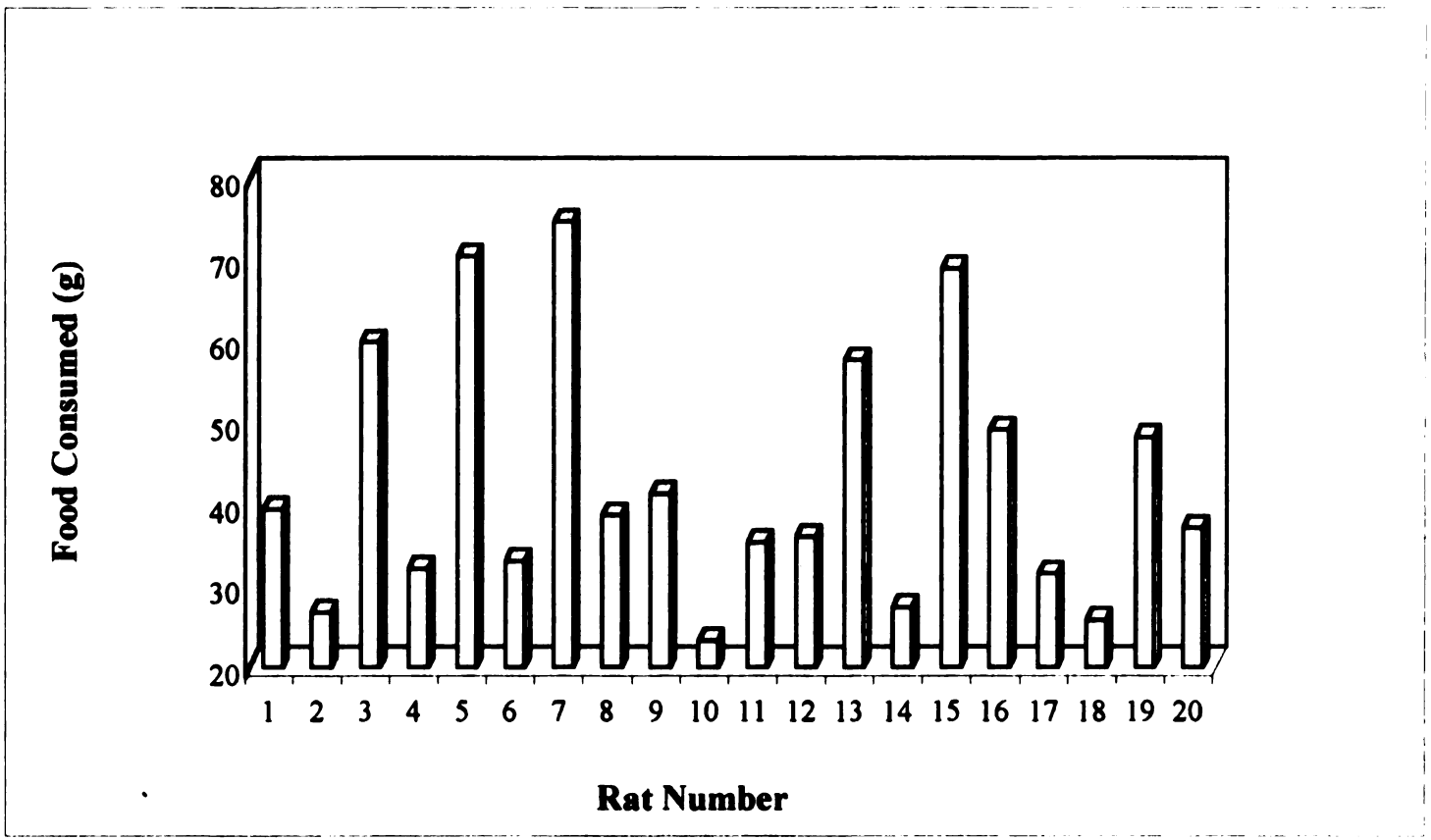

Figure 29 - Food Intake of rats during feeding period 1

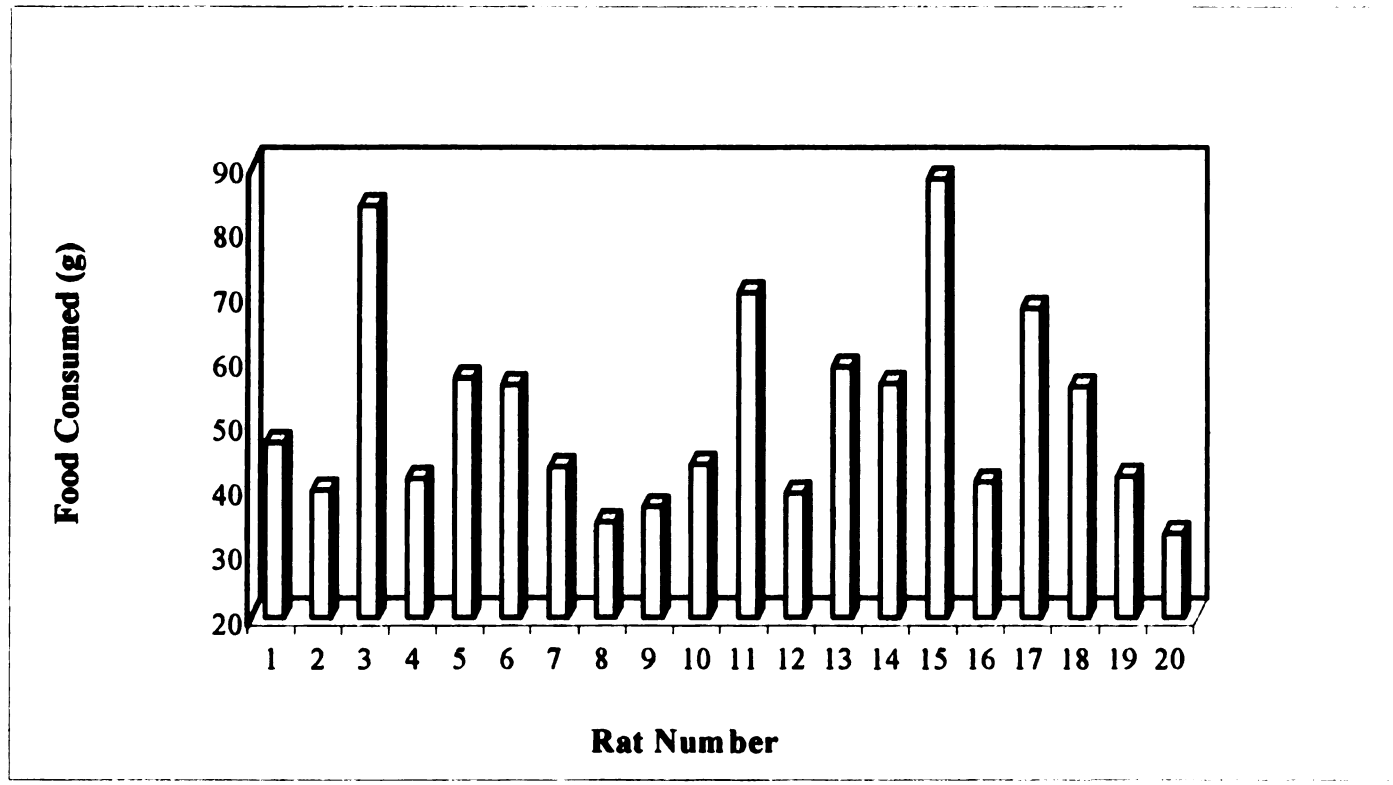

Figure 30 - Food Intake of rats during feeding period 2

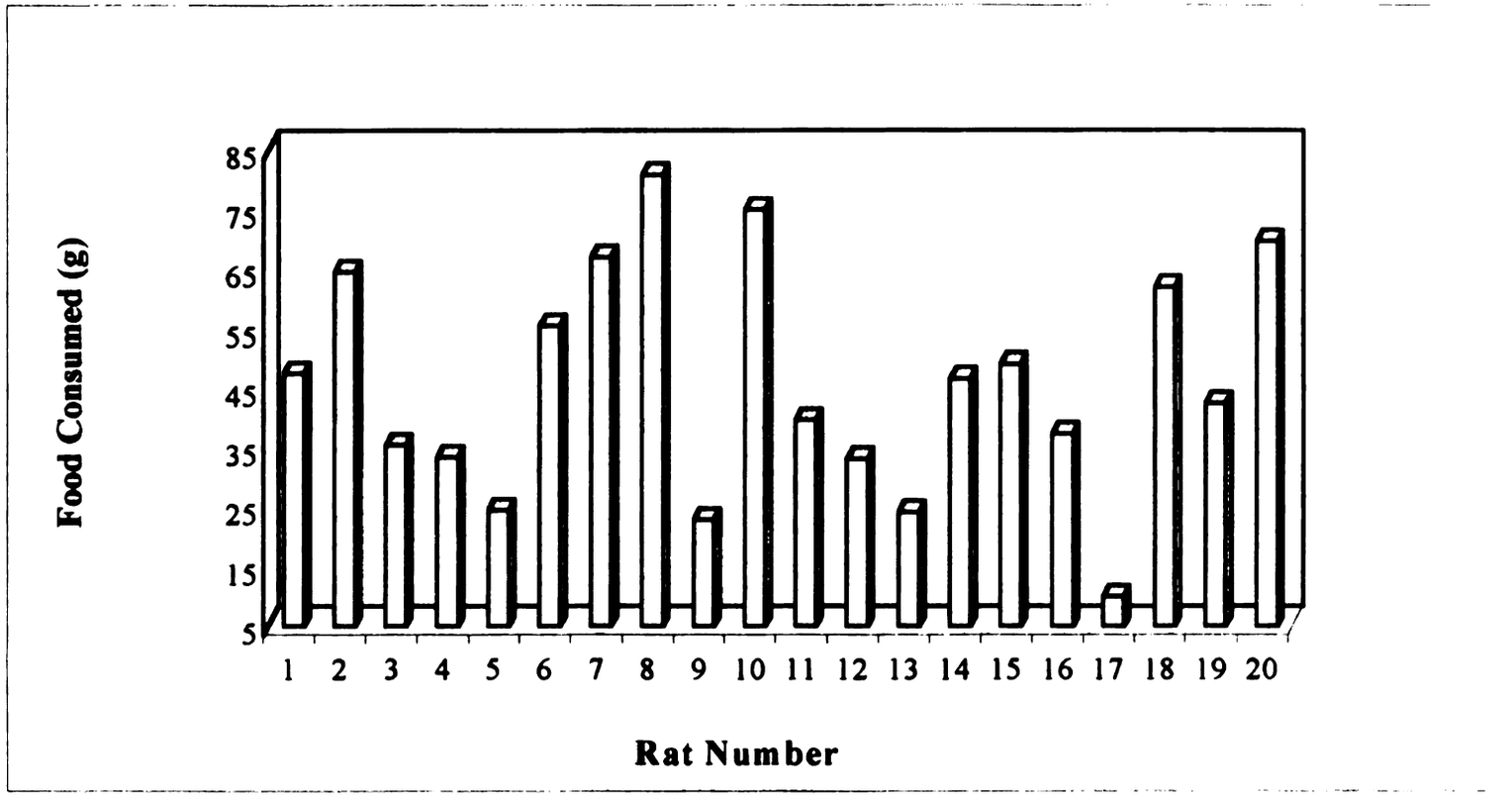

Figure 31 Food Intake of rats during feeding period 3

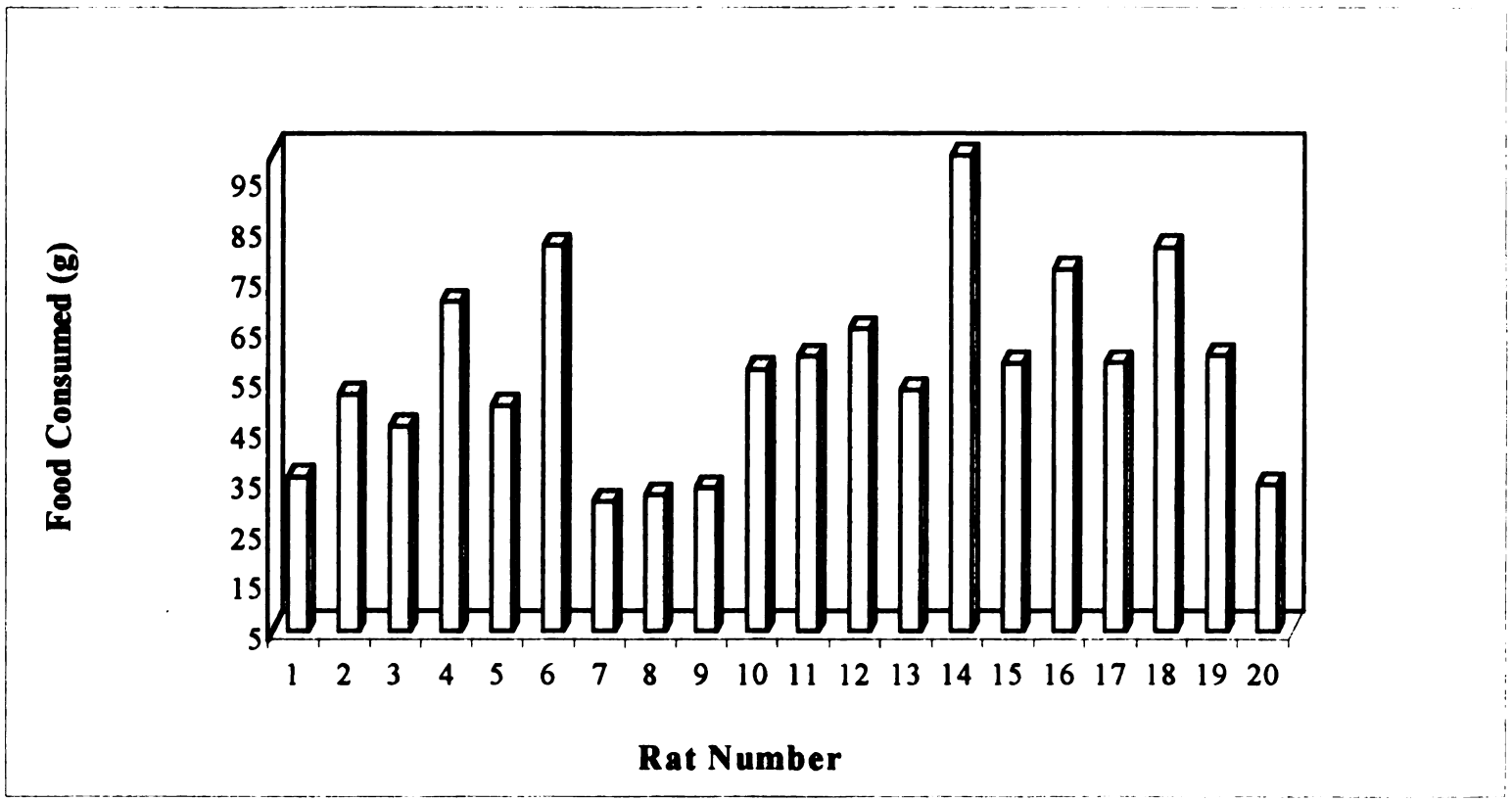

Figure 32 Food Intake of rats during feeding period 4

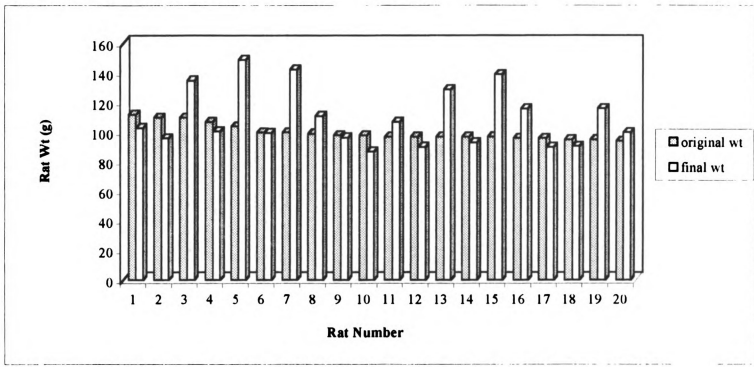

Figure 33 Change in rat weight during feeding period 1

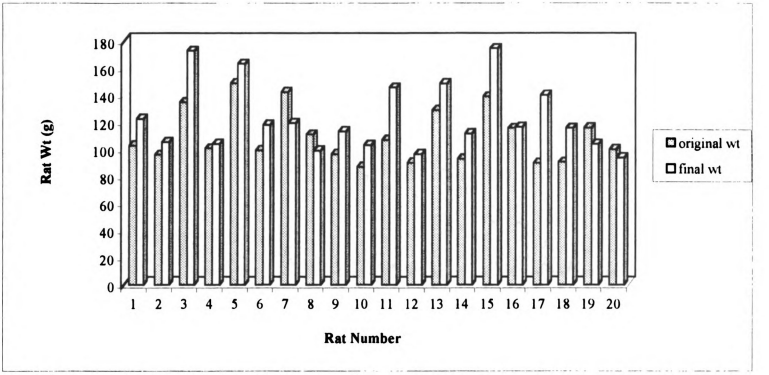

Figure 34 Change in rat weight during feeding period 2

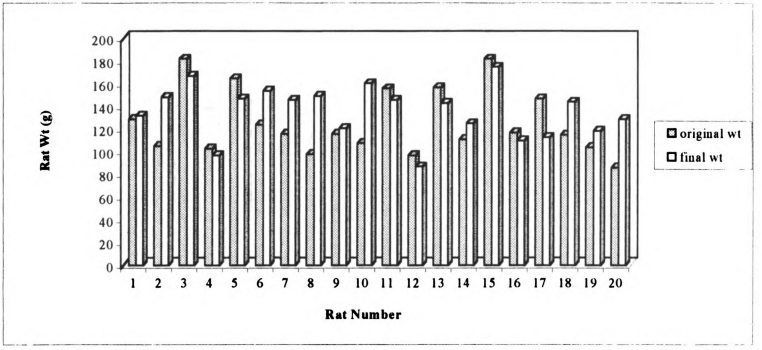

Figure 35 Change in rat weight during feeding period 3

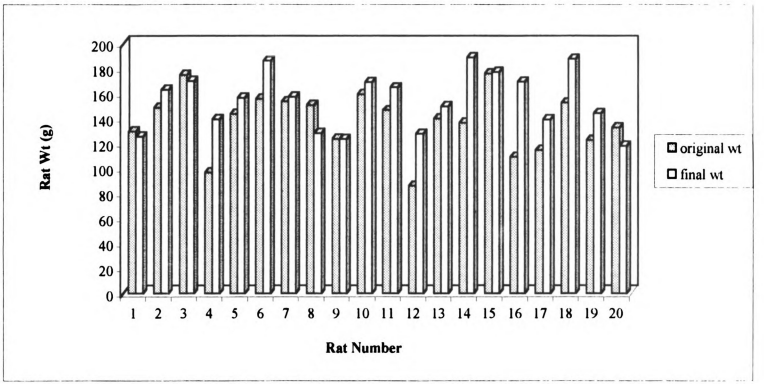

Figure 36 Change in rat weight during feeding period 4

Table 63 Correlation of Protein Nutritional Quality Tests

	RPER	RNPR	RTD	PDCAAS *(in-vivo)*	PDCAAS *(pH-Stat)*	PDCAAS *(pH-Drop)*
RPER	1.00					
RNPR	0.90	1.00				
RTD	0.81	0.75	1.00			
PDCAAS (in-vivo)	0.95	0.84	0.69	1.00		
PDCAAS (pH-Stat)	0.84	0.71	0.41	0.94	1.00	
PDCAAS (pH-Drop)	0.93	0.84	0.57	0.97	0.97	1.00

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