# Production and Functional Characteristics of Protein Concentrates[1]

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**Protein concentrates derived from common dry beans (*Phaseolus vulgaris L.*) may improve world protein resources, reduce on-site preparation time and expense and provide improved nutrition. Several different methods have been studied for the production of these concentrates, including alkali extraction and isoelectric precipitation, ultrafiltration, air-classification and salt extraction under high salt concentrations. Recent studies using solid-solid dry roasting, pin milling and air-classification resulted in the following percent mass fractions: hull/fiber (10%), coarse/starch (70%) and fine/protein (20%). Results indicated that the protein fractions were approximately 45–50% protein, low in raffinose and stachyose and had trypsin inhibitor activity reduced to about half of that of raw beans. Nitrogen Solubility Index (NSI) ranged from 33–70% and was associated with the thermal conditions applied during dry roasting. The flours had a bland flavor without the bitter off-flavors which have traditionally limited the use of dry beans in formulated foods. Most minerals and phytic acid tended to be associated with protein flour; however, although iron may have been bound to phytic acid, its absorption by anemic rats was not hindered by the presence of endogenous phytic acid. These flours produced acceptable products when incorporated into cookies, doughnuts, quick breads and leavened doughs.**

*Characteristics of* Phaseolus vulgaris *proteins.* Common beans contain 20–30% protein on a dry weight basis of which 55–80% are globulins and 10–20% are albumins (1). The quantity of protein in a bean varies among cultivars and commercial classes. For example, Pusztai *et al.* (2) investigated the protein content of ten cultivars of kidney bean and reported that the protein content ranged from 23% for Prelude to 31% for the Cornell cultivar. The amount of globulins present (as a percent of total protein) varied from 62% for the 251, Cornell, 222 and 240 cultivars, down to 55% for Prelude and 196. Thus, there can be a great deal of variability between different cultivars within of the same commercial class of bean.

The proteins in beans can be classified into two categories—metabolic and storage proteins. The storage proteins are the more important of the two since they makeup a higher percentage of the protein in the seeds and are responsible for many of the characteristics of the seed protein fraction (3). The storage (reserve) protein serves to provide a source of nitrogen and carbon compounds for the seedling. The nitrogen is provided as protein, while the carbon is in the form of oil and/or starch (as is the case with *Phaseolus vulgaris*) (4). Several recent studies have shown that the reserve proteins of *Phaseolus vulgaris* are synthesized on the rough endoplasmic reticulum and later accumulated in the protein bodies (5,6). It can be arbitrarily assumed that extracted proteins greater than 5% of the total protein in a seed are storage proteins (4). Beachy (7) extensively reviewed molecular studies which helped define the factors controlling the biosynthesis of seed protein in soybeans, peas, french beans and several varieties of legumes, while methods used for the isolation and characterization of legume storage proteins were extensively reviewed by Derbyshire *et al.* (4).

The isolation and characterization of legume proteins in general and *Phaseolus vulgaris* proteins, in particular, have been the subject of a great deal of research in recent years (8–15), and the work has been summarized in several very extensive reviews (1,4,16,17).

The two major reserve proteins of *Phaseolus vulgaris* are vicilin and phytohemagglutinin, which account for 50% and 10%, respectively, of the total protein content of the cotyledon at maturity (18). Lesser amounts of legumin have been reported in *Phaseolus vulgaris* (4). McLeester *et al* (13) extracted globulin fractions from *Phaseolus vulgaris* and *Vica faba* and subjected them to SDS-gel electrophoresis. This resulted in differing peptide band patterns for these two plants and led the authors to question the usefulness of the terms "legumin" and "vicilin" in the naming of *Phaseolus vulgaris* proteins. The vicilin fraction (which they called G2) appeared to be a non-homogeneous group of proteins.

Investigators at the University of Wisconsin developed the terms G-1 and G-2 to describe the major globulins of *Phaseolus vulgaris* since the features of these proteins do not always correspond to the terms legumin and vicilin as defined by Osborne (11). The G-1 fraction was identified as legumin while G-2 was identified as corresponding to vicilin (13), although there has been some disagreement between researchers about the appropriate nomenclature (18). These authors suggested that the 7S vicilin was identical to the G-1 proteins while phytohemagglutinin is comparable to the G-2 proteins. Also, the term Phaseolin is now used as the trivial name for the G-1 protein (19).

*Phaseolus vulgaris* phaseolin, (or vicilin), is a 7S globulin which forms an 18S configuration at pH 4.5 and has been reported to have between three to five subunits ranging in size from 23,000 to 56,000 (4,5,12,20). Dieckert and Dieckert (21) reviewed the available literature for seed storage proteins in general and concluded that the 7S proteins appear "to be dimers or trimers of the fundamental subunits", and that disulfide bridging between the subunits generally does not occur. However, they also added that "the native

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monomers seem to form associating-dissociating systems depending on the milieu." Very little amino acid sequencing data has been published for these proteins, therefore it is difficult to assess intra-species homology. Derbyshire *et al.* (4), summarized the forms of the 7S proteins from different legume sources for which the characteristics have been determined. Each of these proteins has been reported to have one or two characteristic subunits. However, according to these authors, much work needs to be done to clarify the structure of these proteins. The 7S globulin in *Phaseolus vulgaris* has been determined to be a glycoprotein which contains 4.5% neutral sugars and 1.1% hexosamine (12). Chang and Satterlee (15) isolated and characterized the major protein of Great Northern Beans using classical isolation techniques. The major protein subunits were found in the globulin fraction and had molecular weights of 51,000 and 45,000 with a total molecular weight that was estimated to be 186,000, which would make it a 7S protein. This protein was found to account for 37% of the protein present in the crude bean extract and 31% of the total seed protein. This globular protein was determined to be a glycoprotein which contained 6.5% sugar (as glucose) and contained about 50% alpha-helix. The protein was most stable at pH between 4.0 and 6.0, and was shown to have a compact structure that was resistant to proteolysis.

Phytohemagglutinin (PHA), the lectin of *Phaseolus vulgaris,* has been described (18) as a 6.4S protein with two subunits of molecular weight, 34,000 and 36,000, estimates of PHA's molecular weight range from 115,000 to 150,000 (22). Coffey (22) reviewed seven different studies on the amino acid composition of PHA; these showed that PHA had a large amount of aspartic acid and serine but had practically no cysteine and methionine.

Felsted *et al* (23) investigated the subunit compositions of PHA isoelectins from kidney beans (*Phaseolus vulgaris*). These studies revealed that PHA was comprised of five distinct subunits composed of all possible tetrameric combinations of an erythrocyte reactive subunit (E) and a lymphocyte reactive subunit (L): $E_4$,$E_3L_1$,$E_2L_2$,$E_1L_3$,$L_4$.

Legumin or the G-2 protein is not as well characterized as the other two, but appears to have a sedimentation coefficient of about 11S and has a molecular weight of 340,000. It is a glycoprotein which has an isoelectric point of pH 4.7 (8).

The occurrance of anti-nutritional proteins of both legumes and cereals, their physical and chemical properties and their physiological significance both in plants and in humans has been reviewed by Gatehouse (24). This included the PHA of *Phaseolus vulgaris.*

Bollini and Chrispeels (18) confirmed that the G-1 and G-2 proteins are reserve proteins for the seedling. Seedling growth over a period of 11 days was accompanied by a decrease in the amount of both proteins. The amount of the original polypeptide decreased while the proportion of smaller molecular weight polypeptides increased. Isolated protein bodies from the beans were found to contain the two polypeptides with a combined molecular weight of 60,000.

Dieckert and Dieckert (21) used amino acid data from various sources to statistically compare the relatedness of pairs of proteins, each from a different seed using the difference index (DI). The critical significance values for this index were developed by Cornish-Bowden (25).

Using the DI, they were able to show that generally all of the basic and acidic subunits of legumins from various sources demonstrate relatedness. They also compared amino acid composition data from convicilin of peas, $\alpha$ and $\alpha'$ subunits of $\beta$-conglycinins of soybeans and $\alpha$-conarachin of peanuts. All except $\alpha$-conarachin and $\beta$-conglycinin pass a weak test for relatedness. However, data is lacking to test for relatedness of vicilin proteins in the range of 45-55 kD across species. Thus, it is possible that the major reserve proteins of vegetable seeds have a common ancestry. Also, it is possible to predict structure of unknown seed proteins from proteins with similar physical characteristics in species where the protein structure is known. However, the results of McLeester *et al.*, (13), indicated distinct differences between varieties of beans.

Dieckert and Dieckert (21) noted that while most vicilin-type proteins are glycoproteins, some are not. They speculated that the presence of covalently-bonded oligosaccharides may not be a requirement for biochemical functionality. Rather, this structure may be a relic of an ancestral protein which did require a structure of this type.

Millerd (26) has extensively reviewed the biochemistry of legume seed proteins while Higgins (27) reviewed the synthesis and regulation of the major proteins in seeds including legumes.

*Methods of producing protein concentrates and isolates.* Over the past twenty years, there has been a great deal of interest in the production of protein concentrates from *Phaseolus vulgaris* and other varieties of legumes. Much of this was done to improve the nutritional composition of commonly consumed foods or to provide a functional ingredient for formulated foods.

*Isoelectric precipitation.* One of the commonly used methods has been solubilization of dehulled bean flour in an alkali solution (usually pH 7.0-10.0) and centrifugation followed by isoelectric precipitation of the protein at pH 3.5-4.5. The protein fraction is typically washed several times, neutralized to pH 7.0 and then freeze-dried.

Fan and Sosulski (28) used an isoelectric precipitation method to produce isolates from nine different legume flours, including *Phaseolus vulgaris.* The authors produced isolates which ranged from 92-93% protein for soybean, lupine, and faba bean to 82-83% for pea bean (*Phaseolus vulgaris*), lentil and chickpea.

Kon *et al.* (29) produced legume (*Phaseolus vulgaris*) powders from California small white (CSW) and pinto beans. The beans were ground and added to eight volumes of water, which was acidified to produce a slurry of about pH 3.5. The solution was boiled during addition to prevent clumping. After cooking for 15 min, the slurry neutralized to pH 6-7 and was drum dried. This powder was referred to as the acidified powder. A "regular" powder was also produced in which beans were soaked and cooked for 1 hr in four volumes of water, slurried and then drum dried. The two powders possessed almost identical bulk densities after

the drum dried flakes were ground. The viscosity of a slurry from the acidified powder was less than that for the "regular" powder. The consistencies of slurries from the regular powder decreased rapidly with dilution, but the acidified slurry retained its viscosity at lower concentrations. Nutritional qualities of both bean powders were similar to those of whole cooked beans as determined by animal weight gain, PER values and protein digestibilities. Both powders were free-flowing, non-hygroscopic and convenient to use.

Alli and Baker (30) isolated proteins from *Phaseolus vulgaris* using a citric acid extraction at various pH as well as using the alkali extraction method of Fan and Sosulski (28). The extracted proteins were then examined under a light microscope. The citric acid tended to produce bipyramidal crystalline and spheroidal protein structures while the sodium hydroxide procedure produced amorphous protein structures.

Alli and Baker (31) examined the structures of proteins isolated by citric acid and sodium hydroxide under the Scanning Electron Microscope. The proteins were isolated using the methods previously described (30). Examination of the proteins revealed that several types of particulate structures were present in the citric acid extracted proteins while the sodium hydroxide proteins had only a coral-like structure.

Musakhanian and Alli (32) produced protein concentrates from *P. vulgaris* (white kidney and navy beans) and lima beans using the isoelectric precipitation method of Fan and Sosulski (28) and also precipitated the proteins using citric acid at pH 5.5 and 3.5. They then determined the molecular weights of the protein fractions obtained from the extract, the precipitate and the supernatant of the precipitate of both the citric and the alkali extraction. The authors found that both the alkali and the acid extracts had a fraction of 230,000 molecular weight, which was the major component of the precipitate. The lower weight fractions tended to be found in the supernatant, although the precipitate of the navy bean contained an 18,000 MW fraction which was present only in trace amounts in the kidney precipitate. There appeared to be no real difference between protein fractions which had different microscoped structures.

The lima bean showed different results where the acid extraction gave a precipitate which had a predominant molecular weight of 700,000, while the alkali extracted proteins had a predominate molecular weight of 270,000. Since the acid extracted proteins had a bipyramidal crystalline structure while alkali extracted proteins had an amorphous structure, this suggested a difference in composition between protein structures. The authors concluded that the composition of the extracts from which the protein is precipitated does not affect the composition of the resulting concentrate. Also, differences in chromatographic behavior are related more to genetic differences than to differences in microstructure.

*Salt extraction.* Satterlee *et al.* (33) produced a flour from Great Northern Beans using a hammermill and then extracted the proteins overnight in 2% NaCl. This was followed by centrifugation at 9000 × *g* and dialysis of the supernatant for 48 hr. The precipitated proteins were then centrifuged at 9000 × *g* and the pH of the supernatent was lowered to 3.5. The supernatent was considered to be the albumin fraction while the precipitate was the globulin fraction. These were studied separately and were also recombined to form a bean protein concentrate.

Chang and Satterlee (34) developed a process for producing a bean protein concentrate from a mixture of five varieties of cull beans. The concentrate was produced by extraction of bean flour with a 0.2% salt solution, followed by precipitation at pH 4.0 and various temperatures. A PER study of the various concentrates indicated that the concentrate obtained by acid precipitation at room temperature had a higher nutritional quality than the concentrate which was acid precipitated at 90°C.

Sathe and Salunkhe (35) used a salt solubilization of the bean flour followed by dialysis and freeze-drying to obtain a protein isolate, a protein concentrate, and also the isolated albumins and globulins from the bean protein. The protein content of the concentrate produced by this method was 85.4%, while that of the isolate was 92.4%. Sathe *et al.* (16) believed that this process could be modified to use ultrafiltration rather than dialysis.

They discovered that 0.5% $Na_2CO_3$, 5% $K_2SO_4$, 5% SDS and 0.02N NaOH were the best solubilizing agents for bean proteins. These agents all solubilized 93.6 g of Lowry protein per 100 g of Kjeldahl protein.

SDS-PAGE of the bean flour, concentrate and isolate showed the presence of 22, 14 and 11 subunits, respectively, the same three products showed predominant subunits with molecular weights of 294,000, 146,000 and 135,000 daltons, respectively. Isoelectric focusing indicated that the flour contained 15 subunits, the concentrate had 16 subunits and the isolate had 11 subunits.

*Air-classification.* In the process of air-classification, whole legumes are dry milled into flour and then separated by particle size and density (using an air-classifier) into high starch fraction and high protein fractions. Increased yields can be achieved with repeated milling and air-classification. The two constraints for this process are a low lipid content in the beans to prevent agglomeration of the four, and carbohydrates in the form of starch to achieve classification (36). This process has been used for the production of high protein flour from navy bean (37,38), California small white beans (39), and pea, northern beans, chickpeas, lima beans, faba beans, field peas, mung beans and lentils (40).

Patel *et al.* (37) fractionated navy bean flour in an air-classifier and attained a yield of 34.7% for a protein concentrate with a 62% protein content. Aguilera *et al.* (38) also milled and air-classified navy bean flour into high starch and high protein fractions. The high protein fraction contained 43.1% protein. The high protein flours were incorporated into products ranging from cakes and bread to frankfurters, where they were found to function favorably.

Kon *et al.* (39) hammermilled and then air-classified the flour from California small white beans and obtained high protein flours containing 45% protein. Examination of the high protein flour under a light microscope indicated that most of the hull portion was

concentrated in the coarse fraction and that there was very little starch in the protein fraction.

Sosulski and Youngs (40) milled and air-classified proteins from eight different legumes and compared their functional properties with those of soybean and lupine flours. The pea, Great Northern Bean, chickpea, lima bean flours and their protein concentrates gave higher values in the functional property tests while Faba beans, field pea, mung bean and lentil gave higher protein fractionation in the air-classification step. These authors found that high values for oil absorption, emulsification, whippability and foam stability were characteristic of the high protein fractions. Great Northern Beans and pea beans (*Phaseolus vulgaris*) gave air-classified protein fractions of 55% and 50% protein, and 3% and 4% lipid, respectively, and both types had 1.4% starch.

Tyler *et al.* (41) used the same general procedure for dehulling, milling and air-classifying flours from several types of legumes, including Great Northern and navy beans to form high protein fractions. The percentage of the total flour protein recovered in the protein fraction gave a measure of protein separation efficiency (PSE). Navy and Great Northern beans produced PSE values of 80% and 87%, respectively.

Sahasrabudhe *et al.* (42) pin-milled navy bean cotyledons and air-classified the resulting flour in an Alpine microplex air-classifier. The protein rich fraction was 31-35% of the total by weight and contained 50.3-57.5% protein. Nitrogen solubility at pH 7.5 ranged from 80.1-83.0%, depending upon the bean variety. Water hydration capacity values of the protein fractions did not vary significantly among varieties, but was lower than other vegetable products. The amino acid composition was similar to that of whole beans in that it had a high lysine and low sulfur amino acid content, while the lipids and oligosaccharides were concentrated in the protein-rich flour.

The process of milling followed by air-classification to obtain high protein flours has been reviewed by Sosulski (43). The most extensive research into the use of air-classification for the production of high protein products from *Phaseolus vulgaris* was a project sponsored by the USDA and conducted jointly at Michigan State University and Texas A&M University. This project focused exclusively on the production and utilization of dry edible flour products from *Phaseolus vulgaris.* The flour production process involved three basic steps: (i) dry beans were rapidly heated to 120°C in a solid-to-solid heat exchanger to inactivate antinutritional factors and stabilize flavor; (ii) roasted beans were either pin-milled to produce whole flour or cracked by corrugated rollers to aid in the removal of hulls by air aspiration; and (iii) the cracked cotelydons were pin-milled to a fine flour which was further air-classified into high protein and high starch flours. This procedure was used to produce high protein, high starch and high fiber flours from navy, black and pinto beans. The dry roasting process and the flour production and air-classification processes have been described in detail (44, 45).

*Classification of the flour fractions.* The characteristics of the Navy high protein fraction were evaluated by Zabik *et al.* (46). The high protein navy flour was found to have a composition which was relatively constant regardless of the processing conditions (moisture, 6.1-7.0% [db]; ash, 4.5-7.3%, protein, 39.3-47.6%; fat, 2.7-3.2%; dietary fiber, 2.5-4.5%). Increases in roasting temperature and time were found to significantly reduce the solubility of the protein. The sugar content was determined by high performance liquid chromatography and it was found that stachyose was the only major oligosaccharide in the fraction.

When the high protein flour from pinto beans was analyzed, it was found that this fraction contained 50.8% protein, which was twice the 23.6% protein found in the whole bean (46). The NSI of the protein in the high protein flour was 61.9%, as compared to that of the pinto cotyledon, which was 44.9%.

Tecklenburg *et al.* (47) found that the phytic acid was concentrated in the protein fraction by the air-classification procedure. The authors also found a strong correlation between protein content and the amount of zinc, iron, potassium and magnesium. The correlations between the three suggested that the minerals were present in the protein flour as metallic phytates. Naczk *et al.* (48) found that phytates were primarily associated with the proteins in field peas. Total phosphorous was thought to be a reliable indicator of phytate content because of the presence of phytate phosphorous. When the high protein flour was fed to anemic rats, it appeared that adsorption of iron by anemic rats was not hindered by the presence of phytic acid (49).

The effects of dry roasting and air-classification on protein content, SDS-PAGE, and *in vitro* digestibility for both the navy and pinto high protein flours were evaluated (49). Roasting decreased the protein content of selected fractions; however, the SDS patterns of the salt soluble proteins were similar in the raw and roasted flours. The protein digestibility of the starch and protein fractions for both types of beans were similar and amino acid analyses indicated that no major partitioning occurred among the fractions.

*Storage stability.* The storage stability of the whole navy bean flour, as well as the various fractions, was determined as part of the investigation (50). The equilibrium moisture content (EMC) of the whole flour decreased with increased roasting time and temperature. This effect increased as the relative humidity of the storage increased. Similar effects of roasting conditions on EMC were seen in the other fractions. The high protein fraction had highest EMC, while the low protein hull flour had the lowest EMC at all humidity levels. In all flour fractions, the NSI and sugar content decreased with increasing relative humidity. Generally, the NSI values for flours stored at 20°C under 6 and 9% moisture were unchanged after 24 months.

*Functional properties of* Phaseolus vulgaris *protein products.* Hermansson (51) defined functional properties as those "physico-chemical properties which give information on how a protein will behave in a food system". The functional properties reflect complex interactions between the composition, structure, conformation, physicochemical properties of the proteins, other food components and the nature of the environment in which these are associated or measured. Kinsella (52) extensively reviewed the functional properties of proteins in foods and also summarized some of the

general classifications of protein functional properties which are important in food systems.

Sathe and Salunkhe (53) investigated some of the functional properties of the bean products produced by their salt extraction procedure. These included viscosity, gelation emulsification ability and foamability.

The concentrate and isolate had comparable viscosities at corresponding concentrations, but were more viscous than either the separated albumins or separated globulins at a given concentration. This suggested that combining the albumins and globulins produced substances with different properties than when they were separate.

When the gelation ability of the products was tested, the least concentrations necessary to achieve gelation were 10% for the flour, 85% for the concentrate and 12% for the isolate. This suggested that gelation was not only a function of protein concentration but also the type of protein and nonprotein component(s).

The protein concentrates were shown to readily absorb oil and water while the whole flour was least effective. The isolate was very similar to the flour in both water and oil absorption capacity.

The concentrate was found to have both the highest emulsion capacity and the highest foam forming capacity among all three products, and was better than egg albumin in foam forming ability. However, the stability of foams formed by the bean products was less than that of the egg. All samples showed increasing foamability as protein concentration increased, with a maximum being reached at 10% (w/v) solids.

Sathe and Salunkhe (54) investigated the functional properties of the bean products produced by salt solubilization, dialysis and finally freeze-drying. The bean flour, concentrate and isolate were found to have similar moisture sorption isotherms in which maximum absorption occurred at 4°C while minimum absorption occurred at 38°C. The equilibrium moisture content of the flour was higher, possible due to the presence of carbohydrates in the flour.

The protein products had only modest buffer capacity at pH from 4.0–8.0. Modification of the proteins by succiylation or oxidation increased the oil absorption capacity of all three protein products and the water absorption capacity of the flour and isolate. However, modification of the concentrate decreased its water absorption.

When the adhesiveness of the products was tested, that of the concentrate was found to be about half that of the flour, while that of the isolate was approximately 1/6 that of the flour.

The amino acid composition, *in vitro* digestibility and the performance of these bean protein products in cookies was evaluated (55). The flour and the isolate had high amounts of acidic amino acids, while the concentrate was high in hydrophobic amino acids. The sulphur amino acids and leucine were the first and second limiting amino acids, respectively. All of the products had low *in vitro* digestibility which was improved by heating, with moist heat being more effective than dry heat. Finally, it was noted that there was a decrease in cookie spread ratio as the protein content of the cookies increased.

Satterlee *et al.* (33) determined both the emulsifying and the whipping characteristics of the protein fractions and the concentrate produced by their salt extraction method. The emulsifying characteristics of the proteins were considered to be fair; the albumins had good emulsion capacity but poor stability while the opposite was true for the globulins. The albumins were found to have a good foaming capacity and stability at a concentration of 5% and a pH of 4–5. The globulins were not as effective at foaming but did have slightly better foam stability.

High protein navy and pinto flours were produced by pin-milling and air-classification in the USDA collaborative bean flour utilization project (49). The emulsification capacity of both of these flours was then evaluated. It was reported that the amount of oil which could be emulsified by the flour decreased as the amount of pinto bean flour increased (46). This was attributed to the oil droplets becoming smaller as the amount of protein increased, resulting in a much larger surface area for protein emulsification.

The emulsification capacity of the navy bean protein flour was evaluated at various pH and salt levels (49). Results showed that emulsification capacity increased as pH increased. Low and high salt concentrations had the same effects on capacity while a medium concentration decreased it slightly.

The pinto high protein flour produced stable, high volume foams from pH 4–7, but the volume decreased significantly at pH 10 (46). It was also found that foam viscosity increased with increasing pH but that increasing salt levels in the foams had no effect on foam volume or viscosity. When the high protein flour was substituted for egg white, foam drainage decreased significantly. This was partly attributed to the water binding capacity of the starch in the flour.

*Applications of* Phaseolus vulgaris *flour and high protein products in food systems.* Legume flours have been tested in a variety of food systems, most of them involving traditional baked products. Bahnassey *et al.* (56) prepared legume flour by dry milling navy and pinto beans and lentils and also prepared protein concentrates from the legume flours using the alkali extraction procedure of Fan and Sosulski (28). When these legume products were compared to durum wheat semolina, it was found that they had significantly higher protein, ash, fiber and fat contents than the wheat. A fortified spaghetti was prepared from blends of legume flour or protein concentrates with a strong gluten durum semolina. Analysis revealed that the protein, ash and fiber contents of the fortified spaghetti exceeded the levels for the control spaghetti. The amino acid composition of spaghetti made with the legume products showed a better balance of lysine and sulfur amino acids than that made solely from wheat. The legume flours and their protein concentrates had a relatively higher level of most amino acids than the durum semolina.

Bahnassey and Khan (57) investigated the rheological, processing and quality of spaghetti fortified with legume flours of their concentrates. The legume products were prepared as described as Bahnassey *et al.* (56). Spaghetti was prepared from durum wheat semolina blended with 3% vital wheat gluten and fortified with 0.5, 10, 15, 20 and 25% legume flour or protein

concentrate. The supplemented spaghetti showed an increase in farinograph water absorption, except at the 25% level, which had a slight decrease. The supplemented spaghetti also showed a decrease in the mechanical tolerance index and shattered earlier than the control spaghetti. The spaghetti which was supplemented with legume products tended to have a decrease in its cooked weight and increase in its cooking loss as the level of fortification was increased. Spaghetti supplemented with up to 10% legume flours or protein concentrates was judged acceptable by a taste panel for all tested parameters; however, the panel tended to prefer spaghetti containing legume flours over the spaghetti containing concentrates. A "beany taste" was reported for spaghetti containing 25% legume flours or concentrates.

Satterlee *et al.* (33) studied the use of their salt-extracted protein concentrate in food systems. When the concentrate was added to bread, it caused a decrease in the loaf volume which corresponded to increasing protein content. When added to sugar cookies, the bean proteins enhanced the width:height ratio.

The potential applications for bean flour in food systems were an integral part of a recent USDA Bean Flour Project (50). Potential use of selected flour fractions are presented in Table 1.

*Applications of navy bean flours.* The whole navy bean flour was tested for its effects in quick breads (58). A high quality pumpkin bread was produced with navy bean flour substituted for 35% of the flour. The resulting bread contained 25% more protein than the control.

Whole navy bean flour was also put into apple spice cake, yeast breads and was used as a substitute for wheat flour in bread (50). Rheological data, baking properties and sensory evaluations indicated the navy bean flour was a good supplement for wheat flour in baking. Also, it appeared that the flour could be used to replace up to 45% of the wheat flour in highly favored baked goods without adversely affecting physical or sensory characteristics. However, yeast bread color, volume and flavor were adversely affected by 20% substitution of navy bean flour for bread flour.

Navy bean flour was also substituted for wheat flour in pumpkin bread up to the 50% level, with 35% being the optimum substitution level (59). The presence of bean flour was found to increase the nutritional quality both in terms of protein quantity and quality.

Bread flour was substituted at 0, 5, 10, 15 and 30% with bean flour and incorporated into Chinese steamed bread (49). Results indicated that increased substitution decreased volume while tenderness increased slightly.

The navy hull fraction was tested for its ability to increase the fiber content of spice flavored layer cakes (60) and sugar snap cookies (61). It was also tested in banana bread and as an ingredient in wheat bread (50).

The high starch navy flour was evaluated for its suitability in extruded bean flour puffs and in formulating refried beans (49). Both applications were judged to be acceptable by consumer taste panels.

The high protein navy fraction was tested for its potential as an additive in comminuted meat products (50). This flour was incorporated into frankfurters at different protein levels. Data in this study indicated that frankfurters with 3.95% protein flour yielded an acceptable product. Frankfurters substituted at 7.9% showed limited promise, while substitution at the 11.9% level produced an unacceptable product.

High protein flour was also incorporated into banana bread and doughnut holes (50). High quality banana bread with 0, 15 and 30% high protein flour was produced. This bread was acceptable to a taste panel at the same level as the unsubstituted control. It was reported that high quality, raised doughnut holes could be produced by substituting 25% of the flour with the high protein flour. Since the protein flour has four times the protein of wheat flour and is high in lysine, it was suggested that the flour could help improve the nutritional quality of snack foods.

High protein flours from navy, pinto and black beans were substituted for all-purpose flour in cake

**TABLE 1**

**Potential Uses of Bean Flour Fractions in Formulated Foods**

| Flour fraction | Major characteristics contributed to food | Potential uses in foods |
|---|---|---|
| Whole bean flour | Protein supplement<br>thickening agent | Soups, sauces, dips<br>Baked products<br>"Master Mix" |
| High fiber (hulls) | Dietary fiber<br>water binding | Baked products |
| High protein | Protein concentrate (improved nutritional quality)<br>water binding | Comminuted meats<br>High protein baked products |
| High starch | Gel structure<br>thickening agent | Puddings<br>Pie fillings<br>Prepared gravies and sauces |

doughnuts at levels of 0-30% (61). Doughnuts with 30% navy or pinto flour spread less during cooking than the control while navy bean protein substituted at the level of 10 and 20% produced more tender doughnuts than those made from the black bean or the control. Doughnuts with a 13% substitution of navy or pinto protein were judged most acceptable by a consumer panel and also had less fat, were softer, and showed less firming after six days storage.

*Application of pinto bean flours.* The protein flours from the air-classification of whole pinto flours along with the whole flour itself were tested in various food systems.

Whole bean cotyledon flour was prepared as a substitute for wheat flour in a "master mix" and used to prepare muffins and pancakes (49). Muffins substituted at a 20% level were slightly less acceptable than those made from non-substituted flour. However, pancakes with 20% substitution scored higher in all categories except flavor than the 0% substituted pancakes.

A standard egg noodle recipe was prepared with 0, 5, 10 and 20% of the standard wheat flour replaced with pinto flour (49). Noodles with bean flour were more tender and slightly darker. Sensory evaluation indicated that increased substitution led to decreased flavor and acceptability.

The high protein flour was incorporated into banana bread and peanut butter cookies (46). The results for the banana bread were mixed, while the results for the peanut butter cookies were generally favorable.

When the high protein bean flour was substituted for wheat flour in doughnuts, the final product was more red in color than the control, while the flavor attributes of the substituted doughnuts were higher than the control. In general, substitution levels of 20-30% of the flour were feasible in all three food systems (46).

Breads were also prepared using blends of wheat flour and pinto flour and bread flour and pinto flour. The breads baked with the wheat flour blend (and 10% pinto flour) had a slight decrease in volume, were more tender and had excellent sensory characteristics. Breads with the bread flour blend had fewer differences in volume and tenderness (46). The results of these application tests have been summarized by Uebersax and Zabik (62).

Protein concentrates and isolates made from *Phaseolus vulgaris* have been shown to have great potential as food ingredients. The proteins themselves are the storage or reserve proteins for the beans. These proteins are synthesized on the endoplasmic reticulum and serve as an amino acid "reservoir" for the growing bean plants.

The proteins can be isolated using a variety of methods. The most common method is alkali solubilization followed by acid precipitation. Other methods which have been investigated include salt extraction and air-classification. Air-classification involves using an air stream to separate coarse and fine particles. The fine particles are the high protein fraction.

These protein products have been examined in a variety of food systems. They have been most commonly used in baked products as a partial substitute for regular flour. In these systems, the concentrates help to increase the protein content of the baked products. Thus, it has been shown that protein products from *P. vulgaris* are highly functional and have the potential for use in a wide variety of applications.

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