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Protein Isolates from Navy and Pinto Beans: Their Uses in Macaroni Products

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Navy and pinto bean flour proteins were extracted with dilute alkali solution. Proteins from the extract were precipitated by adjusting the pH to 4.5 and isolated by centrifugation. The isolated proteins were lyophilized or spray-dried. The protein content of the first extraction was 60.5 and 64.3% for navy and pinto bean isolates, respectively. The lysine content of navy and pinto bean protein isolates was more than 4 times greater than that found in durum wheat semolina. Acceptable spaghetti was prepared by using the protein isolates as a source of protein enrichment in macaroni products. The cooking quality of spaghetti made with semolina and navy bean protein isolate was of better quality than spaghetti made with semolina and pinto bean protein isolate.

The increasing concern for the worldwide shortage of good protein led us to investigate new methods of processing plant proteins for human diets. Legumes are an important source of plant proteins for human consumption. They are especially valuable in providing human diets with a well-balanced amino acid content when mixed with processed cereals. Although legume proteins are low in some essential amino acids, they are the main protein intake in certain parts of the world where animal protein is limited. Legume proteins are also considered to be one of the cheapest and the most convenient high-protein materials to offset the amino acid deficiency of cereal proteins. It is well-known that navy and pinto beans (*Phaseolus vulgaris*) have high protein and high lysine content.

Satterlee and Bembers (1975) extracted protein from great northern beans (*P. vulgaris*) with 2% NaCl. The isolated protein caused a decrease in loaf volume when added to bread while it enhanced the width:height ratio of sugar cookies. Maneepun et al. (1974) reported that lima bean protein quality may be improved for fortification with methinonine in the food formulation. They suggested that more balanced amino acid composition can be obtained when lima bean protein concentrate is mixed with other proteins.

Kakade and Evans (1965) reported that unheated navy beans contain trypsin inhibitor and hemagglutinating activities which are destroyed by heating to 121 °C for 5 min. They also reported that heat treatment increased the protein efficiency ratio (PER) of the navy beans. In one of their earlier works (Kakade and Evans, 1964) they showed that the hemagglutinating activity of the bean is soluble at pH 4.0 and the trypsin inhibitor activity was also present in a soluble form at the same pH. Most of the bean proteins are insoluble and will precipitate at pH 4.0 without hemagglutinating or trypsin inhibitor activities.

Yadav and Liener (1977) also found improved nutritional values by roasting navy beans. They showed that various mixtures with cereal grains produced foods with high chemical scores which were not much different than casein.

Field pea protein has been extracted and its functional properties have been characterized by the Prairie Regional Laboratory and University of Saskatchewan, (1974). The amino acid composition of the protein showed it to be an excellent source of lysine and other essential amino acids except methionine. Pea flour or protein concentrate fortified with a small amount of methionine and blended with wheat flour produced PER's equal to those of casein. Patel et al. (1980) determined the amino acid profiles of navy bean flour and found a high lysine content (7.39%). The bean flour also has low amounts of sulfur amino acids of 3.39%.

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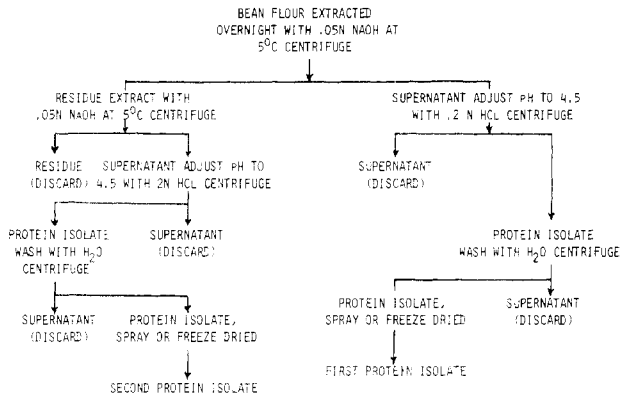

Figure 1. Schematic diagram for alkali extraction of navy and pinto protein isolates from bean flour.

The use of bean flour concentrates and isolates in pasta products has been limited. Lorenz et al. (1979) were able to use up to 20% faba bean concentrates in noodle products which showed acceptable flavor and firmness of the cooked product. Molina et al. (1974) made pasta from hard wheat (*Triticum vulgare*), whole maize, and peeled common beans (*P. vulgaris*). The resulting pasta contained higher available lysine and PER values (3.8 g/16 g of N and 2.02 PER) as compared to those of commercial pasta (1.8 g/16 g of N and 0.97 PER).

The purpose of this investigation was to isolate navy and pinto bean proteins and determine their nutritional contribution to pasta. Their effect on pasta quality was also determined.

EXPERIMENTAL SECTION

Samples. Commercial samples of navy and pinto beans were milled to flour following the procedure described by Watson et al. (1975).

Nitrogen Determination. All proteins and supernatants were analyzed for nitrogen by the American Association of Cereal Chemists method (1962). A factor of 6.25 was used to convert nitrogen to protein in the bean flour, isolate, and residues.

Protein Extraction and pH Profile. Pinto or navy bean flour was dispersed in 0.02 N NaOH solution in a 1:10 flour:solvent ratio and then extracted by stirring overnight at 5 °C. Suspensions were centrifuged at 10000*g* for 15 min at 5 °C.

To determine the pH where the maximum precipitation of protein occurred, 50-mL aliquots of protein extract were adjusted to pH values between 3.0 and 6.5 with increments of 0.5 pH unit by adding 2 N HCL solution dropwise to each flask. The mixture was stirred and then centrifuged for 15 min at 10000*g*. The nitrogen content of the precipitate and supernatant was determined.

Preparation of Protein Concentrates. Protein concentrates were prepared (Figure 1) by suspending a 1000-g sample of navy or pinto bean flour into solution, pH 10.7, 1:10 flour:solvent ratio. The slurry was mixed in a Waring blender for 10 min and then the samples were stirred overnight at 5 °C. The slurry was centrifuged at 10000*g* for 15 min. The supernatant was decanted and adjusted to pH 4.5 by using 2 N HCl for maximum protein precipitation followed by centrifugation. The precipitated protein was lyophilized or suspended in 5 volumes of distilled water and spray-dried. A portion of the residue and the supernatant were analyzed for nitrogen. Residues from the first extraction were subjected to a second extraction using a 1:5 residue:solvent ratio. The first and second isolates (spray- or freeze-dried) were combined for subsequent use.

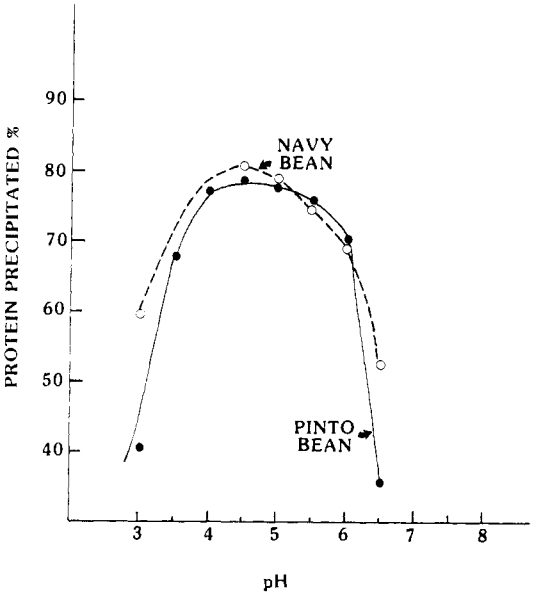

Figure 2. Protein precipitation profile of navy and pinto alkali extractions.

Table I. Protein Content of Alkaline Extraction Products of Navy and Pinto Bean Flours

product	navy, %	pinto, %
flour	24.7	23.7
protein isolate, 1st extraction	60.5	69.3
protein isolate, 2nd extraction	59.1	57.4
residue (after 1st extraction)	4.3	4.8

Nitrogen Solubility. Nitrogen solubility index was determined by mixing 5 g of protein isolates with 50 mL of distilled water and adjusting the pH with either 1 N HCL or 1 N NaOH. Samples were stirred for 20 min and centrifuged at 10000*g* for 15 min. The supernatant was decanted into a 50-mL volumetric flask and diluted to 50 mL and nitrogen was determined.

Amino Acid Analyses. Amino acid analyses were performed by hydrolyzing 30-mg samples in 16 × 17 mm screw-cap tubes with caps having Teflon-faced liners (Kimax 450 77A) at 110 °C for 24 h under N_2 with 3 mL of 6 N HCl containing 2 mol of norleucine internal standard. Amino acids in the hydrolysates were determined by the method of Benson and Patterson (1965) on a Beckman Model 120B amino acid analyzer. Results were adjusted to 100% N recovery from the column. Cystine and methionine were determined as oxidation products (Moore, 1963). Tryptophan was determined on NaOH hydrolysates by using the method of Hugli and Moore (1972).

Experimental Spaghetti Processing. Spaghetti samples were processed in a laboratory unit and spaghetti color, cooked weight, cooking loss, and spaghetti firmness were evaluated as described by Walsh et al. (1971).

RESULTS AND DISCUSSION

Protein Extraction and Precipitation. Figure 2 shows percent protein precipitated at different pH values. Eight pH values between 3.0 and 6.5 in 0.5 increments were used to study maximum protein precipitation. Maximum amount of protein precipitated was 82 and 79% at pH 4.5 for navy and pinto bean alkali extractions, respectively. Therefore, pH 4.5 was used in the procedure to precipitate protein from alkali extracts. Table I gives the protein contents of alkali extraction products. Higher protein was obtained in the navy bean protein isolate than in the pinto bean protein isolate from the first extraction. Protein

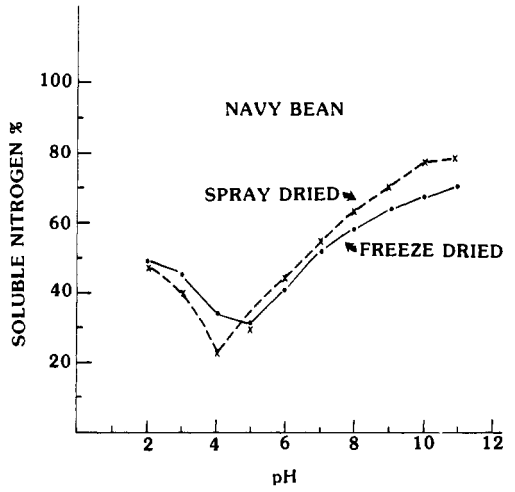

Figure 3. Nitrogen solubility of spray- and freeze-dried navy protein isolates.

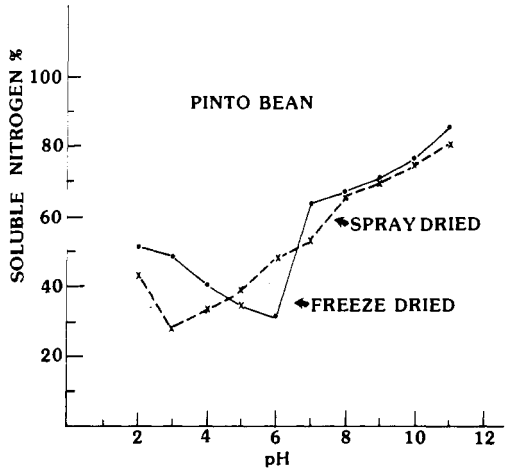

Figure 4. Nitrogen solubility of spray- and freeze-dried pinto protein isolates.

content of the isolate from the second extraction was slightly higher for the navy bean.

Nitrogen Solubility. Nitrogen solubility is affected by the nature and type of protein in the system. It is a function of protein structure, configuration, size, amino acid composition, ionic strength, and pH of the solvent. Figures 3 and 4 show the nitrogen solubility of spray- and freeze-dried (lyophilized) navy and pinto bean protein isolates, respectively. As the pH of the solvent was increased, solubility of the protein isolate decreased to a minimum and then increased again with increasing pH. Minimum solubility of the bean protein isolates was noted for freeze-dried navy at pH 5.0, spray-dried navy at pH 4.0, freeze-dried pinto at pH 6.0, and spray-dried navy at pH 3.0.

Amino Acid Composition. The amino acid compositions of the proteins of navy and pinto bean flours and isolates are listed in Table II and of the fortified durum spaghetti in Table III. The data were calculated to 100% nitrogen recovery and expressed in grams of amino acid per 16 g of nitrogen. Table II shows that lysine content of the bean flour protein was about 4 times the value for durum spaghetti protein as listed in Table III. The total sulfur amino acid content of bean flours was lower than that of the durum protein. Durum spaghetti protein showed higher values for ammonia, glutamic acid, and proline than bean flours, which indicates a gluten-type pattern. In general, most of the other amino acids of bean flour proteins were higher than that of durum protein

Table II. Amino Acid Composition of Navy and Pinto Bean Flours and Protein Isolates[a]

	navy			pinto	
amino acid	flour	SD	FD	flour	SD
lysine	7.94	8.52	9.85	8.04	8.21
histidine	3.02	3.32	3.49	3.14	3.21
ammonia	1.88	2.06	1.55	1.88	1.89
arginine	7.49	6.60	5.53	8.11	7.17
aspartic acid	14.96	14.16	14.47	14.42	14.33
threonine	5.38	4.82	6.19	4.73	4.56
serine	6.50	6.47	7.38	6.12	6.27
glutamic acid	16.49	16.47	16.76	17.35	17.04
proline	4.67	4.58	5.05	4.39	4.64
glycine	4.58	4.40	4.55	4.40	4.34
alanine	4.70	4.23	4.84	4.22	4.04
half-cystine	2.37	2.29	1.31	2.29	2.23
methionine	1.32	1.54	0.70	2.03	1.97
valine	4.80	4.83	7.01	4.74	4.97
isoleucine	4.24	4.44	7.37	3.25	4.46
leucine	8.83	9.29	7.44	8.74	9.11
tyrosine	3.56	4.12	4.32	3.50	4.32
phenylalanine	6.35	6.70	7.08	6.71	6.83
tryptophan	1.03	0.66	0.91	0.85	0.79

[a] Reported in grams per 16 g of nitrogen. SD = spray-dried isolates. FD = freeze-dried isolates.

Table III. Amino Acid Composition of Spaghetti Made from Durum Semolina and from Durum Semolina with Navy and Pinto Protein Isolates plus Vital Wheat Gluten[a]

amino acid	durum semolina (control)	NP + VWG[b]	PP + VWG[c]	FAO pattern[d]
lysine	1.80	5.21	5.18	5.44
histidine	2.46	2.68	2.71	
ammonia	2.67	3.14	3.13	
arginine	4.41	5.46	5.51	
aspartic acid	7.00	9.46	9.80	
threonine	4.20	3.82	3.70	4.00
serine	5.41	5.43	5.46	
glutamic acid	30.39	26.67	26.00	
proline	15.22	8.58	8.87	
glycine	3.42	3.95	3.95	
alanine	3.66	3.53	3.36	
half-cystine	3.15	2.91	2.83	3.52
methionine	1.55	1.08	1.69	
valine	5.86	4.31	4.35	4.96
isoleucine	4.71	3.86	3.99	4.00
leucine	8.89	8.26	8.35	7.04
tyrosine	3.12	3.45	3.52	6.08
phenylalanine	6.56	6.45	6.38	
tryptophan	0.87	0.83	0.89	0.96

[a] Reported in grams per 16 g of nitrogen. [b] 77% semolina, 20% navy protein isolates, and 3% vital wheat gluten. [c] 77% semolina, 20% pinto protein isolates, and 3% vital wheat gluten. [d] *FAO Nutr. Meet. Rep. Ser.* (1973).

Table IV. Amino Acid Scores of Proteins from Durum Wheat Spaghetti and from Durum, Bean Isolates, and Vital Wheat Gluten Spaghetti

amino acid	durum (control)	durum, navy bean isolate, and vital wheat gluten blend	durum, pinto bean isolate, and vital wheat gluten blend
lysine	33	96	95
threonine	105	96	93
half-cystine/ methionine	134	113	128
valine	118	87	88
isoleucine	122	95	100
leucine	126	117	119
tyrosine/ phenylalanine	159	162	163
tryoptophan	91	86	93

Table V. Quality of Spaghetti Made from Semolina and from the Formulas with Bean Protein Isolates

spaghetti	color score	cooked wt, g/10 g	cooking loss, %	firmness score	protein,[a] %
semolina	8.5	36.6	4.4	5.59	12.8
semolina-navy-gluten	8.0	32.3	7.0	5.89	24.3
semolina-pinto-gluten	5.5	30.1	7.0	6.85	24.8

[a] 14% moisture basis.

except valine and phenylalanine, which were lower in bean flour proteins.

The amino acid composition of the spray-dried (SD) and freeze-dried (FD) navy bean protein isolates is listed in Table III to illustrate the effect of heat on the isolates. The differences observed in the amino acid patern of the SD or the FD isolate are in agreement with De Groot (1963) and may reflect the effect of drying method on each amino acid (Table II).

Twenty percent of each isolate was mixed with 77% semolina and 3% vital wheat gluten (VWG) and processed into spaghetti. The vital wheat gluten was used to improve the spaghetti firmness. The amino acid composition of the processed spaghetti is shown in Table III. The Food and Agriculture Organization of the United Nations (1973) amino acid patterns are shown in the table for comparison.

The nutritional quality of proteins is usually related to the amino acid content. Because a high correlation (r = 0.86) was obtained between biological value and amino acid deficits, chemical score (amino acid value/FAO pattern value of the same amino acid) may be the most logical method of predicting protein quality (Block and Mitchell, 1947). The amino acid chemical scores are summarized in Table IV. Both navy and pinto formulas showed improved chemical scores over that of the control spaghetti. The chemical score of the spaghetti made from navy and pinto protein isolates showed 90% or more of the FAO pattern except for the amino acid valine, which had 87 and 88% of the FAO pattern for navy and pinto protein isolates, respectively. The amino acid or chemical score of durum semolina was 33 based upon lysine, which was the first limiting amino acid.

Quality of Spaghetti Made from Bean Protein Isolates. Table V depicts the quality data on the spaghetti processed from 100% semolina and from the bean protein isolates formulas. High pasta color and firmness scores are major quality factors considered by the U.S. consumer. The control sample gave a color score of 8.5 (9.0 or over is considered to be the best), while spaghetti from navy and pinto formulas gave a score of 8.0 and 5.5, respectively. Although the pinto formula gave the lowest color score, it may be considered acceptable when compared to some commercial products. Cooked weights of the spaghetti made from the bean formulas were lower than that of the control. Cooking losses of the navy and pinto formulas were 7.0% compared to 4.4% for the control. A cooking loss of 9.0% or over would be considered unsatisfactory. Firmness scores for bean-fortified spaghetti showed improvement over the durum wheat spaghetti. The quality of the spaghetti made from navy bean protein isolates was acceptable when compared with that of the durum spaghetti. Spaghetti made from the pinto bean formula was judged to be inferior to the durum wheat pasta because of inferior color and low cooked weight. The protein content of both bean formulas was over 24.9% while the control sample contained 12.8% protein.

This study indicates that protein isolates from navy and pinto beans can be mixed with durum wheat semolina to improve the lysine content of the processed product. Mixed with semolina, spaghetti was produced with high protein content from navy and pinto protein isolates.

Registry No. L-Lysine, 56-87-1.

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