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Production and Characterization of a Protein Concentrate from Navy Beans (*Phaseolus vulgaris*)

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ABSTRACT

Different methods were evaluated for the production of a navy bean protein concentrate (NPC). Most of the concentrates prepared by isoelectric precipitation contained 80–83% protein. Salt fractionation of the navy bean flour resulted in low concentrate yields. The most satisfactory method for production of a NPC from dry bean flour was a modification of the isoelectric precipitation method of Fan & Sosulski (1974) in which the centrifugation speed was increased to 15 000 × g. This concentrate had a protein content of 83·9% and a starch content of 2·4%. The concentrate contained more soluble protein and less phytohemagglutinin than the flour from which it was produced. The major protein was a 7S protein with three subunits of about 45–48 kDa typical of vicilin. About 10% of the protein present was in the form of a 60 kDa fraction.

INTRODUCTION

Dry beans (*Phaseolus vulgaris*) are important sources of protein in the Third World, especially in Central and South America. The beans are rich in

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lysine but are deficient in certain amino acids, most notably methionine (Carpenter, 1981). Another problem with beans is the presence of antinutritional factors, such as lectins. When native lectins are fed to laboratory animals, poor growth, diarrhea and death results (Liener, 1986). Cooking beans inactivates much of the lectin, although varying levels of lectin activity are found in normally prepared beans (Rea *et al.*, 1985).

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

The Food and Nutrition Service of the USDA defines a vegetable protein concentrate as one which is not less than 65% protein (N × 6·25), but less than 90% protein. An isolate contains not less than 90% protein. Different methods for the production of soybean isolates have been reviewed by Kolar *et al.* (1985), while those used for the production of concentrates have been reviewed by Campbell *et al.* (1985).

Isoelectric precipitation is a common method for the production of protein isolates from soybeans (Meyer, 1971; Kolar *et al.*, 1985). Fan & Sosulski (1974) used an isoelectric precipitation method to produce isolates from nine different legume flours, including dry beans. The authors produced isolates which ranged from 92–93% protein for soybean, lupin, and fababean and concentrates of 82–83% protein from pea bean, lentil, and chickpea. Bean proteins are very soluble at alkaline pH values and tend to become more soluble as the pH increases (Evans & Kerr, 1963; Hang *et al.*, 1970; Fan & Sosulski, 1974). Centrifugation removes most of the plant material which is insoluble at alkaline pH values. The solution is further purified by reducing the pH to 4·5 which is the point of minimum solubility for the proteins. The proteins are precipitated by centrifugation and dried for storage. Other methods for extracting proteins from legumes, such as salting out, have also been proposed by different researchers (Ma & Bliss, 1978; Murray *et al.*, 1981).

In this investigation, different methods were evaluated for the production of a protein concentrate from navy beans. The concentrate from what was determined to be the 'best' method (simplest procedure, highest protein content and maximum yield) was characterized using proximate analysis, gel electrophoresis and densitometer scans.

MATERIALS AND METHODS

Concentrate production

Michigan navy beans (*Phaseolus vulgaris*) were purchased from a local supplier. The beans were cracked, dehulled by aspiration and ground through a 050 mesh screen into flour using a hammer mill. Protein concentrates were prepared from the flour using procedures described in Table 1. Process yield was calculated by dividing the weight of freeze dried concentrate by weight of navy bean flour and multiplying by 100. Concentrates were stored desiccated at −20°C. All concentrates were prepared in triplicate.

Compositional analysis

The protein content of the navy bean flour and all concentrates was determined using AOAC Method 24.038-24.040 (AOAC, 1984). Moisture was determined using AACC Method 44-40 (AACC, 1983). Ash was determined using a modification of AACC Method 08-01 (AACC, 1983). The crucibles containing dried samples from the moisture determinations were wet ashed by saturating the samples with HCl, digested until a gray ash formed and heated overnight at 550°C. The composition of the navy protein concentrate (NPC) was determined on a dry weight basis. The soluble protein content of the concentrate and navy bean flour were determined in 0·2M NaCl, pH 7·0 using the method of Morr *et al.* (1985). The starch content of the concentrate was determined by hydrolyzing the starch to glucose using the method of Englyst *et al.* (1983). Glucose content of the digested samples was determined using the method of Budke (1984).

Electrophoresis

Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was performed on 12% (0·25% bis) acrylamide running gels with 4% stacking gels using the system of Laemmli (1970). Electrophoresis was performed with a Hoeffer Vertical Electrophoresis unit (Model SE 600; Hoeffer Scientific Instruments, San Francisco, CA) using a constant voltage power supply (Heathkit Model 1P-17, Benton Harbor, MI). One hundred micrograms of navy bean and flour concentrate protein and 35 μg of the soluble flour and concentrate proteins were applied to the gel. A constant current of 30 mA was applied until the proteins migrated into the running gel and then the current was increased to 60 mA until the bromophenol blue

TABLE 1
Methods Evaluated for the Production of Navy Bean Concentrate

Method number	*Process description*
1.	*Fan & Sosulski* (1974): Extract bean flour for 1 h in 0·02% NaOH; centrifuge 15 min at 1 500 × *g*; extract residue for 1 h in 10 vol. of 0·02% NaOH; centrifuge; combine supernatants; adjust pH to 4·5 using conc. HCl; centrifuge; wash residue and freeze-dried to 3% moisture.
2.	*Alkaline Extraction*: Performed using Method 1 except first extraction performed for 24 h.
3.	*Alkaline Extraction*: Same as Method 1 except 15 000 × *g* centrifugation used.
4.	*Alkaline Extraction*: Same as Method 1 except first extraction performed for 3 h and 15 000 × *g* centrifugation used.
5.	*Alkaline Extraction*: Performed using Method 1 except 20 000 × *g* centrifugation used.
6.	*Defatting of Navy Bean Flour*: Bean flour was defatted using Goldfisch ether extraction and then extracted using Method 1.
7.	*Acetone Extraction*: Protein extracted using Method 1. Precipitated proteins were resuspended in 0·02% NaOH and then precipitated using cold acetone.
8.	*Salting Out*: Extract 15 *g* flour in 100 ml of 1·7% NaCl solution for 30 min at 37°C; centrifuge 5 000 × *g* for 10 min; lower pH of supernatant to 4·5; centrifuge; freeze dry pellet.
9.	*Ma & Bliss Salt Extraction*: Bean flour extracted using salting out procedure of Ma & Bliss (1978).
10.	*Sathe & Salunkhe (1981) Extraction*: Extract proteins using 0·5% Na_2CO_3; centrifuge 10 000 × *g* for 30 min; dialyze supernatant 48 h at 4°C; centrifuge; freeze dry pellet.
11.	*Whole Navy Bean Extraction*: Whole beans soaked for 12 h in H_2O. Beans ground in Waring Blender for 2 min in 1:5 solution of 0·02% NaOH: beans. Proteins extracted at pH 8·5 for 3 h. Solution centrifuged at 20 000 × *g* for 30 min; supernatant filtered through glass wool and No. 52 Whatman filter paper. Proteins precipitated at pH 4·5 using 0·05 N HCl with precipitation timed to occur for 15–30 min. Solution centrifuged for 30 min at 20 000 × *g*.

tracking dye reached the bottom of the running gel. The gels were removed and stained for 6 h in 0·125% Coomassie Blue in 50/10/40 (v/v/v) methanol/acetic acid/water. The gels were destained in 7/5/88 (v/v/v) acetic acid/methanol/water until clear.

The relative mobility (RM) of each protein subunit was calculated and the molecular weight (MW) was estimated from a standard curve of RM vs log MW prepared using a mixture of low molecular weight (14·2–

66 kDa) proteins (Sigma Chemical Corp., St Louis, MO). The relative quantities of vicilin, PHA and 60 kDa protein expressed as a percentage of total protein in each treatment were determined by scanning densitometry at 580 nm using a Shimadzu Dual Wavelength Thin-Layer Chromato Scanner (Model cs-930, Kyoto, Japan). The protein bands were identified by their subunit molecular weights.

Statistics

Basic statistics, analysis of variance (ANOVA) and difference testing were performed using M-STAT (1988, Version 4·0, Michigan State University, East Lansing, MI). Two way ANOVA was performed to test significance within replications and between treatments. Mean separations (significance) were tested using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Production of a navy bean protein concentrate

The protein content and yield of each concentrate production method is summarized in Table 2. Most of the concentrates prepared by isoelectric precipitation had protein contents around 80–83% (N × 6·25) except for the acetone extracted material (Method 7) which had a protein content of 90·7%. This method was considered unsuitable for production of a functional concentrate as the proteins were insolubilized by the acetone. Salt fractionation of the navy bean flour (Methods 8, 9) resulted in very poor yield of NPC. No improvements in protein content or process yield were observed when the bean flour was defatted (Method 6) prior to isoelectric precipitation. The use of Methods 8–11 resulted in concentrates containing less than 80% protein. No significant differences in protein content or process yield were observed for Methods 3 through 6. Method 3 was selected for further study as Method 4 specified a longer extraction time, Method 5 a higher centrifugation speed and Method 6 required additional preparation steps. Method 3 extracted approximately 50% of the original flour protein.

Composition of navy concentrate

The composition of the flour and concentrate derived from the modified isoelectric precipitation procedure of Fan & Sosulski (1974) is shown in Table 3. Protein content of the concentrate was over three times that of the flour. There was less starch and ash in the concentrate as compared to the

TABLE 2
Protein Content and Process Yields of Navy Protein Concentrates

Method	*Protein* (%)	*Process yield*[a] (%)
1	81·7[e]	14[e]
2	83·1[e]	10[c,d]
3	83·9[e]	16[e,f]
4	84·0[e]	16[e,f]
5	83·1[e]	18[f]
6	82·5[e]	18[f]
7	90·7[f]	10[c,d]
8	70·7[c]	7[c]
9	70·5[c]	6[c]
10	62·7[b]	<1[b]
11	77·1[d]	10[c,d]

[a] Process yield

$$= \frac{\text{freeze-dried weight of concentrate (g)}}{\text{weight of flour (g)}} \times 100$$

[b–f] Means in the same column followed by the same letter were not significantly different at $p < 0{\cdot}05$.

composition of the dry bean flour. Tomkinson (1986) reported that navy bean flour contained 44·6% starch. Extraction increased the soluble protein content by 6·7%. This increase in protein solubility may be due to increased water dispersibility of the proteins caused by freeze drying or selection of the most soluble proteins in the bean flour by the extraction procedure.

Electrophoresis

The photograph of the SDS–PAGE slab gel (Fig. 1) indicates vicilin and phytohemagglutinin (PHA) and most of the minor proteins were present in the same relative proportions in both the navy bean flour and concentrate. Vicilin was the most common protein in the soluble flour and concentrate preparations. These empirical results are confirmed by densitometer scans of the gel slabs (Table 4).

The vicilin fraction, which is the major protein fraction in navy beans, comprised approximately 44% and 54% of the total protein in the flour and concentrate, respectively. There is also a protein fraction in both the flour and concentrate which has a molecular weight of about 60 kDa. The presence of this protein fraction was noted by Bollini & Chrispeels (1978) and Barker *et al.* (1976). Derbyshire & Boulter (1976) isolated a fraction from

TABLE 3
Composition of Navy Flour and Concentrate[a]

	Concentrate (%)	*Flour* (%)
Moisture	2·7	6·1
Protein	85·4	27·2
Soluble protein	71·1	64·4
Ash	2·6	4·1
Starch	2·4	44·6[b]

[a] Dry Weight Basis. Means are the average of triplicate determinations.
[b] Value from Tomkinson (1986).

dry beans with a molecular weight of 62 kDa which they identified as a breakdown product of an 11S protein. In the flour, the 60 kDa fraction comprised about 10% of the total protein, slightly more than the amount of PHA present. In the NPC, the 60 kDa fraction comprised about 10% of the total protein present while the amount of PHA decreased to about 4·6%. This loss in PHA was accompanied by an increase in the amount of vicilin in

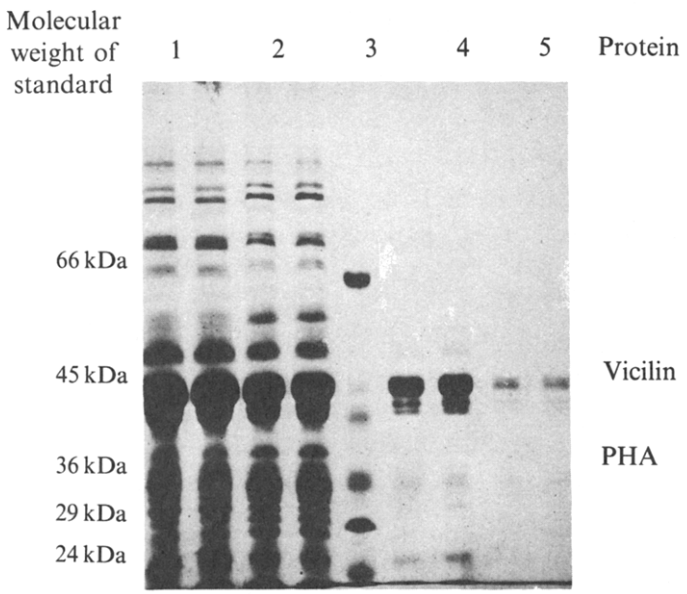

Fig. 1. Representative sodium dodecyl sulfate–polyacrylamide electrophoregram of navy bean flour and concentrate (1 = navy protein concentrate; 2 = navy bean flour; 3 = molecular weight standards; 4 = soluble concentrate proteins and 5 = soluble flour proteins).

TABLE 4
Relative Protein Composition of Navy Concentrate, Flour, Soluble Concentrate Protein and Soluble Flour Proteins Determined by Densitometer Scans of Electrophoresis Gels

Treatment	*Protein fraction (percentage of total protein in each treatment)*		
	60 kDa	*Vicilin*	*Phytohemagglutinin*
Flour	10·5	44·3	9·2
Concentrate	10·2	54·8	4·6
Soluble flour	0·0	42·0	7·2
Soluble concentrate	2·7	75·1	3·3

the concentrate. Isoelectric precipitation may provide a method for reducing the antinutritional factors in beans by reducing the amount of PHA present.

The major soluble protein in the concentrate and flour was vicilin. The 60 kDa protein was not soluble in the flour, but comprised a small percentage of the concentrate soluble protein. The 60 kDa protein may have been partially extracted from an insoluble complex in the flour during preparation of the concentrate.

CONCLUSIONS

The most satisfactory method for production of large amounts of a protein concentrate from dry bean flour was a modification of the isoelectric precipitation method of Fan & Sosulski (1974) in which the centrifugation speed is increased to $15\,000 \times g$. This concentrate had protein content of 83·9% and a starch content of 2·4%. The concentrate produced by this method contained more soluble protein and less PHA than the flour from which it was produced. The major protein was a 7S protein with three subunits of about 45–48 kDa, typical of vicilin. About 10% of the protein present was in the form of a 60 kDa fraction.

REFERENCES

AACC (1983). *Approved Methods*, 8th edn. American Association of Cereal Chemists, Inc., St Paul, MN.

AOAC (1984). *Official Methods of Analysis*, 14th edn. Association of Official Analytical Chemists, Washington, DC.

Aguilera, J. M., Lusas, E. W., Uebersax, M. A. & Zabik, M. E. (1981). Process development, characterization and utilisation of dry-heated navy bean products—Final Report. Texas A & M, College Station, TX and Michigan State University, East Lansing, MI.

Barker, R. D. J., Derbyshire, E., Yarwood, A. & Boulter, D. (1976). Purification and characterization of the major storage proteins of *Phaseolus vulgaris* seeds and their intracellular and cotyledonary distribution. *Phytochem.*, **15**, 751–7.

Bollini, R. & Chrispeels, M. J. (1978). Characterization and subcellular localization of vicilin and phytohemagglutinin, the two major reserve proteins of *Phaseolus vulgaris* L. *Planta*, **142**, 291–8.

Budke, C. C. (1984). Determination of total available glucose in corn base materials. *J. Agric. Food Chem.*, **32**, 34–7.

Campbell, M. F., Kraut, C. W., Yackel, W. C. & Yang, H. S. (1985). Soy protein concentrates. In *New Protein Foods*, ed. A. M. Altschul & H. L. Wilcke, Vol. 5. Academic Press, Orlando, FL, pp. 302–7.

Carpenter, K. J. (1981). The nutritional contribution of dry beans (*Phaseolus vulgaris*) in perspective. *Food Technol.*, **35**(3), 77–8.

Derbyshire, E. & Boulter, D. (1976). Isolation of legumin-like protein from *Phaseolus aureus* and *Phaseolus vulgaris*. *Phytochem.*, **15**, 411–14.

Englyst, H. N., Anderson, V. & Cummings, J. H. (1983). Starch and non-starch polysaccharides in some cereal foods. *J. Sci. Food Agric.*, **34**, 1434–40.

Evans, R. J. & Kerr, M. H. (1963). Extraction and precipitation of nitrogenous constituents of dry navy beans. *J. Agric. Food Chem.*, **11**, 26–9.

Fan, T. Y. & Sosulski, F. W. (1974). Dispersibility and isolation of proteins from legume flours. *Can. Inst. Food Sci. Technol. J.*, **7**, 256–9.

Hang, Y. D., Steinkraus, K. H. & Hackler, L. R. (1970). Comparative studies on the nitrogen solubility of mung beans, pea beans, and red kidney beans. *J. Food Sci.*, **35**, 318–20.

Kolar, C. W., Richert, S. H., Decker, C. D., Steinke, F. H. & Van der Zanden, R. J. (1985). Isolated soy protein. In *New Protein Foods*, ed. A. M. Altschul & H. L. Wilcke, Vol. 5. Academic Press, Orlando, FL, pp. 260–99.

Laemmli, U. K. (1970). Cleavage of structural proteins during assembly of the head of bacteriophage T-4. *Nature*, **227**, 680–5.

Liener, I. E. (1986). Nutritional significance of lectins in the diet. In *The Lectins. Properties, Functions and Applications in Biology and Medicine*, ed. I. E. Liener, N. Sharon & I. J. Goldstein. Academic Press, NY, pp. 522–7.

Ma, Y. & Bliss, F. A. (1978). Seed proteins of common beans. *Crop Sci.*, **18**, 431–7.

Meyer, E. W. (1971). Oilseed protein concentrates and isolates. *J. Am. Oil Chem. Soc.*, **48**, 484–8.

Morr, C. V., German, B., Kinsella, J. E., Regenstein, J. M., Van Buren, J. P., Kilara, A., Lewis, B. A. & Mangino, M. E. (1985). A collaborative study to develop a standardized food protein solubility procedure. *J. Food Sci.*, **50**, 1715–18.

Murray, E. D., Myers, C. D., Barker, L. D. & Maurice, T. J. (1981). Functional attributes of proteins—A noncovalent approach to processing and utilizing plant proteins. In *Utilization of Protein Resources*, ed. D. W. Stanley, E. D. Murray and D. H. Lees, Food and Nutrition Press, Westport, CT, pp. 158–76.

Rea, R., Thompson, L. U. & Jenkins, D. J. A. (1985). Lectins in foods and their relation to starch digestibility. *Nutr. Res.*, **5**, 919–29.

Sathe, S. K. & Salunkhe, D. K. (1981). Solubilization and electrophoretic characterization of the great northern bean (*Phaseolus vulgaris* L.) proteins. *J. Food Sci.*, **46**, 82–7.

Tomkinson, R. A. (1986). Studies on the indigestible and fermentable components of dry bean and cereal grains. MS Thesis, Michigan State University, East Lansing, MI.