Identification and Characterization of Phaseolin Polypeptides in a Crystalline Protein Isolated from White Kidney Beans (*Phaseolus vulgaris*)

Inteaz Alli,*,† Bernard F. Gibbs,‡ Monika K. Okoniewska,† Yasuo Konishi,‡ and France Dumas‡

Food Science and Agricultural Chemistry Department, McGill University, P.O. Box 187, Macdonald Campus, Ste. Anne de Bellevue, Quebec, Canada H9X 3V9, and Biotechnology Research Institute, National Research Council of Canada, 6100 Royalmount Avenue, Montreal, Quebec, Canada H4P 2R2

A crystalline food protein isolated from dried seeds of white kidney beans (*Phaseolus vulgaris*) was investigated for the presence and characteristics of phaseolin polypeptides. Reversed-phase high-performance liquid chromatography (RP-HPLC) of the crystalline protein gave five fractions, of which the first two eluting fractions (F1 and F2) were found to contain phaseolin polypeptides. Ion-spray mass spectrometry showed that the average molecular weights (MW) for F1 and F2 were 49 615 and 48 075. Amino acid composition and N-terminal sequence analysis indicated that the fractions F1 and F2, separated from the crystalline protein, contained polypeptides which were similar to those reported for α-type and β-type phaseolin precursors, respectively.

INTRODUCTION

Over the years plant proteins have become important functional ingredients in many prepared foods; examples include proteins and their modified products prepared from cereal grains, oil seeds, and legume seeds. In most instances, the proteins prepared from plant sources for food uses represent storage proteins. Procedures used for preparation of these storage proteins for food uses often involve isoelectric precipitation (Paredes-Lopez *et al.*, 1991; Fan and Sosulski, 1974). Several researchers have investigated other isolation procedures in order to prepare food proteins from plants (Murray *et al.*, 1981; Alli and Baker, 1980; Melnychyn, 1969); the aim of these procedures has been to isolate the protein with minimal structural and molecular changes and therefore with retention of functional characteristics. The effects of denaturation due to isoelectric precipitation and the resulting influence on molecular characteristics, e.g., thermal properties (Murray *et al.*, 1981; Paderes-Lopez *et al.*, 1991) and functionality, have been reported (Ma and Harwalker, 1988). Concurrently, there has been increased interest in understanding the structure–functional relationships of these proteins to explain and even predict certain desired characteristics (Song and Damodaran, 1987; Kinsella, 1982, 1981); this requires elucidation of the molecular characteristics of the proteins.

The dried seeds of many legumes have been investigated as sources of food proteins. The major storage protein of seeds of *Phaseolus vulgaris* is phaseolin, or G1 globulin; it is glycosylated mainly with mannose, has a molecular weight of approximately 160 K, and shows pH-dependent association–dissociation behavior between tetrameric, protomeric, and polypeptide forms of the molecule (Romero *et al.*, 1975; Sun *et al.*, 1974). It has been demonstrated by several researchers (Bollini and Vitali, 1981; Hall *et al.*, 1977; Romero *et al.*, 1975; McLeester *et al.*, 1973) that phaseolin is comprised of three different polypeptide units of molecular weights ranging from 43 K to 53 K; the MW ranges reported are as follows: for the largest subunit, 50.5–53 K; for the intermediate subunit, 47–49 K; and for the smallest subunit, 43–46 K. Paaren *et al.* (1987) fractionated, from phaseolin, α-, β-, and γ-subunit components and suggested that differential degree of glycosylation could explain the observed molecular heterogeneity found among phaseolin polypeptides. Although sequencing information on the isolated polypeptides is not available, the sequence of an α-type phaseolin precursor (MW 49 271) and a β-type phaseolin precursor (MW 47 566) have been reported on the basis of translation from DNA and RNA sequences (Slightom *et al.*, 1983, 1985); the α- and β-type phaseolins differed mainly in their glutamic acid, glutamine, and glycine contents.

In previous studies, we have observed that crystalline proteins isolated by citric acid extraction of *Phaseolus* beans were considerably less denatured and more soluble than the noncrystalline isoelectric proteins obtained by alkali extraction from the same beans (DiLollo *et al.*, 1993); the characteristics of these proteins in relation to their food use were considered. The trypsin inhibitory activity of the crystalline proteins was also lower than that of the noncrystalline proteins (Li *et al.*, 1989). In addition, the crystalline proteins were heterogeneous mixed crystals which contained 3–8% carbohydrate, with mannose being the principal sugar (Musakhanian and Alli, 1990), and 0.5–5% phytic acid (Alli and Baker, 1980). In the present study, we have used the crystalline food protein isolated from *P. vulgaris* to investigate the molecular characteristics of the polypeptides of this potential food protein preparation. The aim is to determine the extent to which the characteristics of the crystalline protein were similar to those of native phaseolin through the identification and characterization of phaseolin polypeptides. This could give an indication of the extent of preparation of denaturation associated with the isolation of the crystalline protein which could be used to explain certain functional and nutritional characteristics.

MATERIALS AND METHODS

Preparation of Protein Sample. A bipyramidal crystalline protein preparation was obtained from dried seeds of white kidney beans (*P. vulgaris*) by extraction of the ground seeds with citric acid solution (0.4 N, pH 4.0). The proteinaceous extract was refrigerated (4 °C, 18 h) to precipitate protein material, which

* Author to whom correspondence should be addressed.
† McGill University.
‡ National Research Council Canada.

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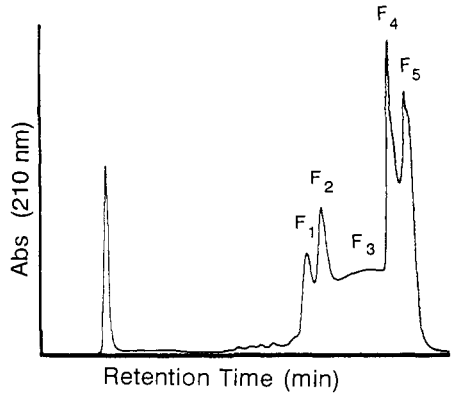

Figure 1. Reversed-phase high-performance liquid chromatography (RP-HPLC) of crystalline protein isolated from white kidney beans (*P. vulgaris*).

was recovered by centrifugation and lyophilized; details of the procedure along with the crystalline structure (light microscope) have been reported previously (Alli and Baker, 1980). The protein content of the lyophilized isolate was determined by the micro-Kjeldahl method (AOAC, 1980).

RP-HPLC Separation of Polypeptides. The lyophilized isolate was subjected to reversed-phase high-performance liquid chromatography (RP-HPLC) to separate the phaseolin polypeptides. The separation was done on a diphenyl reversed-phase column (0.46 × 25 cm length, Vydac 219TP54). A quantity (3 mg) of protein sample was solubilized in trifluoroacetic acid (TFA) solution (1 mL, 0.1%) and injected into a liquid chromatograph equipped with a diode array, UV–visible detector (HP1090, Hewlett-Packard). Elution was done at 1 mL/min using the following two-buffer gradient system: buffer A, 0.1% TFA in water; buffer B, 0.1% TFA in 70/30 acetonitrile/water, starting at 30% B and increasing to 45.5% in 15.5 min, then to 70% B in 1 min, and holding at 70% B for 10 min. The eluate was monitored at 210 μm. The fractionated proteins were collected and dried in a Speed-vac concentrator (Savart, NY) under vacuum. The dried fractions were rechromatographed using the identical conditions described above.

Amino Acid Composition. Approximately 100 μg of protein material was placed in Corning culture tubes (Catalogue No. 9820, 6 × 50 mm) which were previously muffled at 450 °C overnight. The tubes were placed in reaction vials, and the samples were dried in the Waters Pico-Tag work station (Waters, Division of Millipore). Constant boiling HCl (200 μL) containing 1% phenol was added to the vials, which were alternately purged (with dried nitrogen) and evacuated. After three purges, the vials were heated at 150 °C for 1 h and cooled, and the contents of the tubes were dried in a Speed-vac concentrator. The residues were dissolved in sodium citrate buffer, and the analysis was performed on a Beckman System 6300 high-performance analyzer according to the procedures of Spackman *et al.* (1958) as modified by Veeraragavan *et al.* (1990).

N-Terminal Amino Acid Sequence. Automated Edman degradation was performed in a gas-phase sequencer (Model 470A) equipped with an on-line phenylthiohydantoin (PTH) analyzer (Model 120A from Applied Biosystems, Inc.) employing the general protocol of Hewick *et al.* (1981). Samples were applied to TFA-treated cartridge filters coated with 1.5 mg of polybrene and 0.1 mg of NaCl (Biobrene Plus, ABI); a standard program (03RPTH, ABI) was employed for sequencing. The PTH amino acids (PTH-aa) were identified by comparing the retention times with those of the standard amino acid derivatives.

SDS Electrophoresis. SDS gel electrophoresis, according to the procedure of Laemmli (1970), was used for separation of the polypeptide components; 4% stacking gels and 7% running gels of polyacrylamide were used. Slab gels (0.75-mm thickness) were run at 125 V until the tracking dye (bromophenol blue) reached to the 1-cm mark at the bottom of the slab; the electrophoresis was performed with a Bio-Rad Mini Trans blotting system. Protein was detected by staining the gels with Coomassie Brilliant Blue followed by destaining with methanol/acetic acid (50/10 v/v).

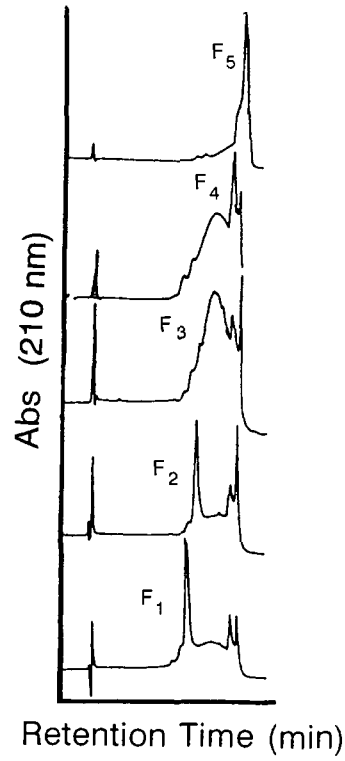

Figure 2. Rechromatography by RP-HPLC of five fractions (F1–F5) recovered from first chromatography of white kidney bean crystalline protein.

Mass Spectrometric Determination of MW. Molecular weights of the crystalline proteins and the purified fractions were determined by ion-spray mass spectrometry using a triple-quadrupole mass spectrometer (API III LC/MS/MS system, Sciex, Thornhill, ON, Canada) according to the procedure described by Feng *et al.* (1991). Multiply charged protein ions were generated by spraying the sample solution through a stainless steel capillary held at high potential. The voltage on the sprayer was set at 5.2 kV for position ion production. A coaxial air flow along the sprayer was provided to assist the liquid nebulization; the nebulizer pressure was adjusted in the range of 25–35 psi. The sample was delivered to the sprayer by a syringe infusion pump (Model 22, Harvard Apparatus, South Natick, MA) through a fused silica capillary of 100-μm i.d. The liquid flow rate was set at 1.0 μL/min for sample introduction. The interface between the sprayer and the mass analyzer was made of a small conical orifice of 100-μm diameter. The potential on the orifice was set at 35 V during calibration and was raised to 80–150 V for proteins to enhance ion signals. A gas curtain formed by a continuous flow (1.2 L/min) of N_2 in the interface region served to evaporate the aerosol droplets and to break up the cluster formation from supersonic expansion. For MW determination, only the first rod set (Q1) of the triple-quadrupole system was used, and the other two (Q2 and Q3) served only as ion guidance lenses. The instrument mass-to-charge ratio scale was calibrated with the ammonium adduct ions of poly(propylene glycols) (PPG). The unit resolution was maintained across the entire mass range for singly charged PPG calibrant ions, according to the 50% valley definition (i.e., in the group of two adjacent peaks the smaller peak was resolved to at least 50% of its full height). The same resolution setting was used for protein MW measurement to avoid possible interference on accuracy due to resolution adjustment. All protein mass spectra shown were obtained from signal averaging of multiple scans.

RESULTS AND DISCUSSION

The protein isolated from the white kidney bean (*P. vulgaris*) for investigation of phaseolin polypeptides contained 95.7% protein and 2.60% total carbohydrate (DiLollo, 1990). Light microscope examination of the protein precipitate before lyophilization indicated that

Table I. Amino Acid Composition of Protein Fractions Separated from *P. vulgaris* Proteins and of Phaseolin Precursors

	detd (mol %)					calcd (mol %)		
amino acid	F1	F2	F3	F4	F5	original protein	α-type phaseolin precursor	β-type phaseolin precursor
Asx	13.0	13.1	13.4	13.4	13.3	13.3	11.7 (51)[a]	12.1 (51)[a]
Thr	3.2	3.0	3.4	3.3	3.6	3.5	3.2 (14)	3.3 (14)
Ser	7.6	7.9	7.0	7.2	7.2	7.4	9.0 (39)	9.3 (39)
Glx	19.1	17.4	17.0	17.8	17.4	18.4	16.1 (70)	14.1 (59)
Gly	6.3	5.2	6.1	6.4	6.3	6.3	6.2 (27)	5.5 (23)
Ala	4.6	4.8	5.4	5.2	5.3	5.3	5.5 (24)	6.0 (25)
Cys	ND	ND	ND	ND	ND	ND	0 (0)	0 (0)
Val	5.2	5.5	5.7	5.8	6.2	5.9	6.5 (28)	6.7 (28)
Met	0.9	0.7	0.5	0.6	0.5	0.7	1.2 (5)	1.2 (5)
Ile	4.9	5.0	5.4	5.6	5.2	4.9	5.8 (25)	6.0 (25)
Leu	9.7	10.1	9.9	9.7	9.6	9.6	10.1 (44)	10.7 (45)
Tyr	2.0	2.2	2.7	2.8	2.6	2.6	2.8 (12)	3.1 (13)
Phe	5.6	5.8	5.7	5.8	5.5	5.6	6.0 (26)	6.0 (25)
His	2.8	2.5	2.6	2.6	2.5	2.6	2.5 (11)	2.4 (10)
Lys	7.6	7.3	6.6	7.2	6.8	5.9	5.8 (25)	5.7 (24)
Arg	4.4	4.4	4.3	3.8	4.1	4.2	4.4 (19)	4.5 (19)
Pro	3.0	4.0	3.9	3.9	3.8	3.8	3.4 (15)	3.6 (15)

[a] Number of residues from amino acid sequence.

Table II. N-Terminal Amino Acid Sequences of Residues 26–36 of Fraction Obtained from *P. vulgaris* Protein

fraction	amino acid sequence
F1	two sequence signals as follows:
	25 26 30 35 36 Signal 1 - T S L R E E E E S Q D Signal 2 - S L R E E E E S Q D N
F2	two sequence signals identical to those of F1
F3	more than two sequence signals, with two sequences identical to those of F1
F4	same as F3
F5	same as F3
α-type[a] and β-type[a] phaseol in precursors	25 26 30 35 36 T S L R E E E E S Q D N

[a] Slighton *et al.*, 1983, 1985.

the microstructure was bipyramidal crystalline (Alli and Baker, 1980; Musakhanian and Alli, 1990). Figure 1 shows the chromatogram obtained from RP-HPLC separation of the isolated protein; five fractions (designated F1–F5) were separated; however, base-line separation of the peaks was not achieved. Attempts to optimize the chromatographic conditions did not result in improved base-line separation. Consequently, the five fractions were recovered and rechromatographed in order that each fraction could be obtained in the relative absence of the adjacent fractions. Figure 2 shows the chromatograms obtained from rechromatography of the five fractions; the results confirm that the fractions obtained from the first chromatography of the original protein also contained adjacent fractions. On the basis of the rechromatography, the five fractions were collected and subjected to further characterization so that it could be established whether any of these fractions in the crystalline protein contained phaseolin polypeptides. Our chromatographic separation is similar to that reported by Paaren *et al.* (1987) who used RP-HPLC to separate two polypeptide fractions from a preparation of phaseolin from *P. vulgaris*; by modification of the separation gradient, these workers obtained a third peak which was considered to be an undissociated promoter phaseolin. We consider that, in our study, fractions 3–5 could represent undissociated proteins in the crystalline isolate.

Table I shows the amino acid composition (mol %) of the five fractions (F1–F5) and the original unseparated protein. The mol % of each amino acid, calculated on the basis of the amino acid sequence of α-type (Slightom *et al.*, 1983) and β-type (Slightom *et al.*, 1985) precursors shown in Table I, indicates that the two polypeptide precursors have similar amino acid compositions except for glutamic acid (and glutamine) and glycine; the α-type phaseolin precursor contained higher amounts of these amino acids when compared with the β-type precursor. Comparison of the amino acid composition of the five fractions obtained indicates that the similarities and differences between fractions F1 and F2 are consistent with those between the α-type and β-type phaseolin precursors, respectively; fraction F1 contained higher amounts of glutamic acid (and glutamine) and glycine than fraction F2. This suggests that the earlier eluting fraction F1 contains a polypeptide which corresponds to the α-type phaseolin polypeptide precursor while the second eluting fraction F2 contains a polypeptide corresponding to the β-type phaseolin polypeptide precursor. Fractions F3–F5 show similar amino acid compositions to the original unfractionated protein with the exception of minor differences in lysine and glutamic acid residues.

Table II gives the N-terminal amino acid sequences for residues 25–35 of the five fractions along with the reported sequences of α- and β-type phaseolin polypeptide precursors. The results show that all fractions give a sequence (sequence I) identical to that of the α- and β-type precursor. Fractions F1 and F2 gave two identical sequences, one of which was missing threonine at position 25. These two sequences were present in all five fractions. The absence of threonine at position 25 in the second sequence (sequence II) suggests that the phaseolin polypeptide undergoes some type of activity, possibly enzymatic, immediately after the signal peptide at position 24, resulting in the loss of threonine. The similarity in observed sequences with that of α- and β-phaseolin precursor supports the suggestion (from amino acid composition) that fractions F1 and F2 contain phaseolin polypeptides and that these polypeptides are also present

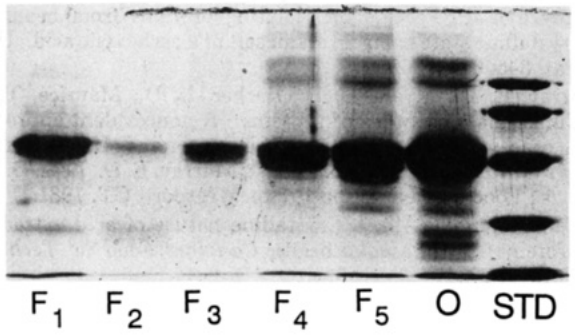

Figure 3. SDS–PAGE of five fractions (F1–F5) and unfractionated crystalline protein (0).

in fractions F3–F5. However, because of the limited sequence determined, the results do not permit an inference as to which of fractions F1 and F2 corresponds to the α-type or to the β-type phaseolin polypeptide.

The SDS–PAGE separation (Figure 3) of the five fractions and the original unfractionated protein shows that the latter contained the phaseolin polypeptides as the major component. The relatively large quantities of these components are likely the cause of the heavy staining and poor resolution. However, fraction 3 is resolved into three polypeptides, and this fraction could represent the undissociated protomeric phaseolin. This agrees with the finding of Paaren *et al.* (1987) who also obtained, using RP-HPLC, a fraction which contained the undissociated protomeric phaseolin. The similarities of fractions F4 and F5 to the unfractionated protein suggest that these two fractions contain the undissociated phaseolin as well as other non-phaseolin protein components which together elute as a single fraction during RP-HPLC. Amino acid analysis (Table I) also suggests the similarity of these fractions with the original protein while the amino acid sequence suggests the presence of non-phaseolin proteins in these two fractions. The characteristics of these fractions (F4, F5) in relation to the original protein merits further investigation. Fraction 1 contains two of the phaseolin polypeptides while fraction F2 shows primarily the lower of the two MW phaseolin polypeptides present in fraction F1; F1 gave two phaseolin polypeptide components with SDS–PAGE in spite of the fact that it was separated as a single component by RP-HPLC. Paaren *et al.* (1987) also showed that the earliest eluting peak from RP-HPLC consisted of α- and β-type phaseolin subunits, with the leading edge of the peak being enriched in the α-component. Although the SDS–PAGE was carried out under conditions which would permit estimation of the MW of the subunits, because of the lack of complete resolution of the phaseolin components, no attempt was made to estimate MW from the SDS–PAGE. However, determination of MW was accomplished by mass spectrometer (MS).

Figure 4 shows the interpreted mass spectra obtained from fractions F1 (a) and F2 (b). The peaks are broad, suggesting a heterogeneity of the glycosylation in both proteins; Paaren *et al.* (1987) also reported that differential glycosylation is largely responsible for much of the molecular weight heterogeneity found among phaseolin polypeptides. F1 shows molecular weights (MW) in the range from 49 290 to 49 940, with an average MW of 49 615, and F2 shows MW in the range from 47 750 to 48 400, with an average MW of 48 075. These values agree with those reported by other workers (Bollini and Vitali, 1981; Hall *et al.*, 1977; Romero *et al.*, 1975; McLeester *et al.*, 1973) for the MW of phaseolin polypeptides. Further intrepretation of the mass spectral data was based on the following

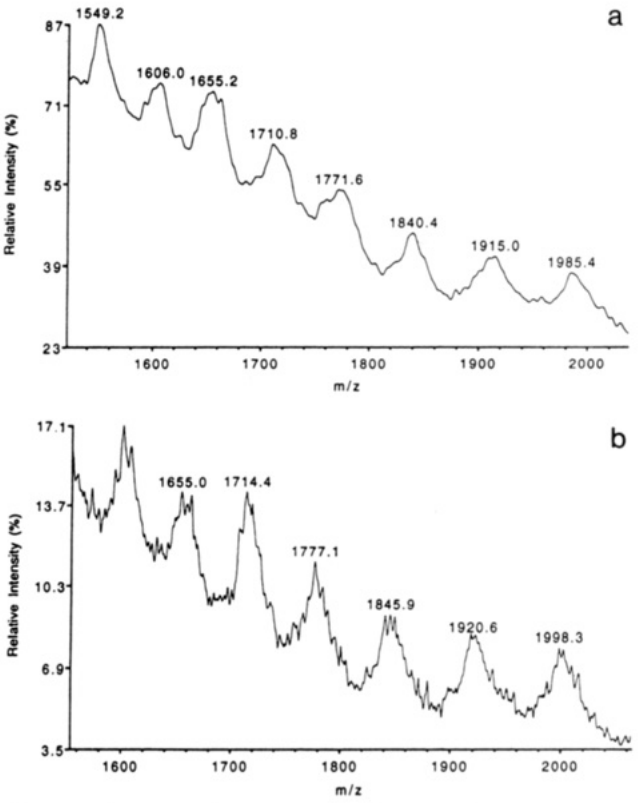

Figure 4. Interpreted mass spectra of fractions F1 (panel a) and F2 (panel b).

two assumptions: (i) fractions F1 and F2 are comprised predominantly of α-phaseolin and β-phaseolin, respectively (suggested from amino acid analysis and denaturing gel electrophoresis), and (ii) N-terminal Thr^{25}, which was a major N-terminal amino acid over Ser^{26} in protein sequencing, dominated the signals in mass spectra. Then, F1 (containing α-phaseolin) has 16–20 mannose units with an average of 18 mannose units, and F2 (containing β-phaseolin) has 17–21 mannose units with an average of 19 units. Since α- and β-phaseolins have identical sequences at around the potential glycosylation sites (Asn^{258} and Asn^{347} for α-phaseolin and Asn^{252} and Asn^{341} for β-phaseolin; Slightom *et al.*, 1983, 1985), a similar degree of glycosylation is reasonable; however, it is not clear why β-phaseolin has an extra mannose compared to that of α-phaseolin. Further studies to confirm these assumptions and more detailed analysis of glycosylation are in progress.

CONCLUSION

The present work has shown that a crystalline protein prepared from dried seeds of white kidney beans (*P. vulgaris*) contains at least two phaseolin polypeptides. The polypeptides were separated by RP-HPLC and identified following amino acid composition and sequence and mass spectrometric molecular weight determination and subsequent comparison with the cited characteristics of α-type and β-type phaseolin polypeptides. Further work is in progress to use the crystalline protein to separate the phaseolin polypeptides on a preparative scale in order that their functional characteristics can be evaluated.

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LITERATURE CITED

Alli, I.; Baker, B. E. Constitution of leguminous seeds: VIII. The microscopic structure of proteins isolated from *Phaseolus* beans. *J. Sci. Food Agric.* **1980**, *31*, 1316–1322.

AOAC. Automated Kjeldahl method for total nitrogen determination. In *Offical Methods of Analysis of the Association of Official Analytical Chemists*; Horwitz, W., Ed.; AOAC: Washington, DC, 1980.

Bollini, R.; Vitale, A. Genetic variability in charge microheterogeneity and polypeptide compositions of *Phaseolus vulgaris*; and peptide maps of its three major subunits. *Physiol. Plant.* **1981**, *52*, 96–100.

DiLollo, A. Thermal and surface properties of crystalline and non-crystalline legume seed proteins. M.Sc. Thesis, McGill University, Montreal, Canada, 1990.

DiLollo, A.; Alli, I.; Biliarderis, C.; Barthakur, N. Thermal and surface active properties of citric acid extracted and alkali extracted proteins from *Phaseolus* beans. *J. Agric. Food Chem.* **1993**, *41*, 24–29.

Doyle, J. J.; Schuler, M. A.; Godette, W. D.; Zenger, V.; Beachy, R. N.; Slightom, J. L. The glycosylated seed storage proteins of Glycine max and *Phaseolus vulgaris*. Structural homologies of genes and proteins. *J. Biol. Chem.* **1986**, *261*, 9228–9238.

Fan, T. Y.; Sosulski, F. W. Dispersibility and isolation of protein from legume flours. *Can. Inst. Food Sci. Technol. J.* **1974**, *7*, 256–259.

Feng, R.; Konishi, Y.; Bell, A. W. High accuracy molecular weight determination and variation characterization of proteins up to 80 ku by Ion Mass Spectrometry. *J. Am. Soc. Mass Spectrom.* **1991**, *2*, 387–401.

Hall, T. C.; McLeester, R. C.; Bliss, F. A. Equal expression of the maternal and paternal alleles for the polypeptide subunits of the major storage protein of the bean *Phaseolus vulgaris* L. *Plant Physiol.* **1977**, *59*, 1122–1124.

Hewick, R. M.; Hunkapiller, M. W.; Hood, L. E.; Dreyer, W. J. A gas–liquid solid peptide and protein sequenator. *J. Biol. Chem.* **1981**, *256*, 7990–7997.

Kinsella, J. E. Functional properties of proteins: Possible relationships of food proteins. In *Food Proteins. Food Chem.* **1981**, *7*, 273–288.

Kinsella, J. E. Relationships between structure and functional properties of food proteins. In *Food Proteins*; Applied Science: London, 1982.

Laemmli, U. K. Cleavage of structural proteins during the assembly of the head of Bacteriophage T4. *Nature* **1970**, *227*, 680–685.

Li, Z.; Alli, I.; Kermasha, S. Tryptic hydrolysis (*in vitro*) of crystalline and non-crystalline proteins from *Phaseolus* beans. *J. Agric. Food Chem.* **1989**, *37*, 643–647.

Ma, C. Y.; Harwalker, V. R. Studies of thermal denaturation of oat globulin by differential scanning calorimetry. *J. Food Sci.* **1988**, *53*, 531–534.

McLeester, R. C.; Hall, T. C.; Sai-Ming, M. Sun; Bliss, F. A. Comparison of globulin proteins from *Phaseolus vulgaris* with those from *Vicia faba*. *Phytochemistry* **1973**, *2*, 85–93.

Melnychyn, P. Process for isolating proteins from beans in crystalline state using an alkali salt of a carboxylic acid. U.S. Pat. 3450688, 1969.

Murray, E. D.; Myers, C. D.; Barker, L. D.; Maurice, T. J. Functional attributes of proteins—A noncovalent approach to processing and utilization of plant proteins. In *Utilization of Plant Proteins*; Stanley, D. W., Murray, E. D., Lees, D. H., Eds.; Food and Nutrition Press: Westport, CT, 1981.

Musakhanian, J.; Alli, I. Crystalline nature of acid extracted proteins from *Phaseolus* beans. *Can. Inst. Food Sci. Technol. J.* **1990**, *23*, 47–52.

Paaren, H. E.; Slightom, J. L.; Hall, T. C.; Inglis, A. S.; Blagrove, R. J. Purification of a seed glycoprotein: N-terminal and deglycosylation analysis of Phaseolin. *Phytochemistry* **1987**, *26*, 335–343.

Paredes-Lopez, O.; Ordovica-Falomir, C.; Olivares-Vasquez, M. R. Chick-pea protein isolate. Physicochemical, functional and nutritional characterization. *J. Food Sci.* **1991**, *56*, 726–729.

Romero, J.; Sai-Ming, M. Sun; McLeester, R. C.; Bliss, F. A.; Hall, T. C. Heritable variation in a polypeptide subunit of the major storage protein of the bean, *Phaseolus vulgaris* L. *Plant Physiol.* **1975**, *56*, 776–779.

Slightom, J. L.; Sun, S. M.; Hall, T. C. Complete nucleotide sequence of a French bean storage protein gene: phaseolin. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 1887–1901.

Slightom, J. L; Drong, R. F.; Klassy, R. C.; Hoffman, L. M. Nucleotide sequences from phaseolin cDNA clones: the major storage proteins from *Phaseolus vulgaris* are encoded by two unique gene families. *Nucleic Acids Res.* **1985**, *13*, 6483–6498.

Song, K. B.; Damodaran, S. Structure–function relationship of proteins: adsorption of structural intermediates of bovine serum albumin at the air–water interface. *J. Agric. Food Chem.* **1987**, *35*, 236–241.

Spackman, D. H.; Stein, W. H.; Moore, S. Automatic recording apparatus for use in the chromatography of amino acids. *Anal. Chem.* **1958**, *30*, 1190–1206.

Sturm, A.; Van Kuik, A. J.; Vliegenthart, J. F. G.; Chrispeels, M. J. Structure, position, and biosynthesis of the high mannose and the complex oligosaccharide side chains of the bean storage protein Phaseolin. *J. Biol. Chem.* **1987**, *262*, 13392–13403.

Sun, S. M.; McLeester, R. C.; Bliss, F. A.; Hall, T. C. Reversible and irreversible dissociation of globulins from *Phaseolus vulgaris* seed. *J. Biol. Chem.* **1974**, *249*, 2118–2121.

Veeraragavan, K.; Colpitts, T.; Gibbs, B. F. Purification and characterization of two distinct lipases from *Geotrichum candidum*. *Biochim. Biophys. Acta* **1990**, *1044*, 26–33.

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