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Tryptic fragments of phaseolin from protein isolates of *Phaseolus* beans

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Abstract

Crystalline and amorphous protein isolates from white kidney and navy beans (*Phaseolus vulgaris*) and baby lima and large lima beans (*Phaseolus lunatus*) were subjected to tryptic hydrolysis. The hydrolysis was monitored by the nitrogen solubility index (NSI) and reverse phase-high performance liquid chromatography/mass spectrometry (RP-HPLC/MS) analysis of the hydrolysates. The results of the NSI and RP-HPLC showed that the tryptic fragments of the crystalline isolates possessed higher solubility properties than the fragments of the amorphous isolates. The results of the MS study of phaseolin peptides in the RP-HPLC fractions of the hydrolysates showed that trypsin-specific bonds located in β-structured regions of β-phaseolin are resistant to tryptic hydrolysis, and that the most susceptible bonds to trypsin hydrolysis are located in regions not directly involved in the formation of secondary structural units, but in regions of disordered structure, or in regions interconnecting secondary structural units. Some trypsin-specific bonds located within α-helical structures were also cleaved by trypsin hydrolysis.

1. Introduction

Proteins have become increasingly important as functional ingredients in many formulated foods as a result of a greater understanding of their physicochemical, nutritional and functional characteristics. The use of chemical and enzymatic techniques to improve the physicochemical properties and functionality of protein isolates have been the subject of several reports (Huang & Kinsella, 1987; Chobert, Bertrand-Harb & Nicholas, 1988; Campbell, Shih & Marshall, 1992; Datta, Figueroa, Oriana & Lajolo, 1993). Proteolytic modification of food proteins involves the use of selected peptidases or proteases which may be specific or non-specific to cleave peptide bonds along the polypeptide backbone. This cleavage results in the formation of small to medium size molecules, and an increase in hydrophilicity due to the formation of extra amino and carboxyl terminal groups. Limited proteolysis is also accompanied by other physicochemical changes, which can alter protein functionality through subunit dissociation, or unfolding of the compact structure of native protein to expose their interior hydrophobic regions.

In general, limited proteolysis increases the solubility (Chobert et al., 1988; Turgeon, Gauthier & Paquin, 1992), dispersibility and diffusion of proteins, thereby providing an entropic force towards increased surface activity (Song & Damodaran, 1987). Interest in the utilization of proteins and their hydrolysis products by the food industry has extended to plant sources, such as soybeans and other legumes. One of the main factors affecting the utilization of plant proteins is their resistance to proteolysis, which in turn reduces the biological availability of their constituent amino acids (Lienier & Thompson, 1980). This finally has lead to several investigations aimed at understanding both the structural and functional changes that accompany the hydrolysis of these proteins.

Storage proteins of common bean, *Phaseolus* sp., consists of 11.5–31% albumins and 46–81% globulins. Phaseolin, a 7S globulin is the major storage protein of *P. vulgaris*, accounting for over 50% of the total seed proteins. It is an oligomeric protein, consisting of three polypeptide subunits; α-, β-, and γ-phaseolin (Romero,

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Saai-Ming, McLeester, Bliss & Hall, 1975; Hall, McLeester & Bliss, 1977; Bollini & Vitale, 1981). The molecular weights of the subunits range from 43 to 53 kDa. The subunits also display molecular heterogeneity, which has been attributed to differential degrees of glycosylation (Paaren, Slightom, Hall, Inglis & Blagrove, 1987). The sequence of the α and β subunits have been reported (Slighton et al., 1985). Recently, the X-ray structure of phaseolin has also been solved at 2.2 Å resolution (Lawrence, Izard, Beuchat, Blagrove & Colman, 1994).

Like most legume proteins, phaseolin is resistant to hydrolysis by several proteolytic enzymes. It, however, differs from other legume proteins in that its hydrolysis by trypsin and pepsin stops after a few susceptible peptide bonds have been cleaved (Lienier & Thompson, 1980; Jivotovskaya, Vitalyi, Vitalyi, Horstmann & Vaintraub, 1996). The unique hydrolysis properties of phaseolin compared with other 7S proteins has been attributed to structural peculiarities of phaseolin (Jivotovskaya et al., 1996). Its digestibility and functional properties have also been studied (Deshpande & Damodaran, 1989ab). Other studies have been directed at different preparation methods of plant protein concentrates and isolates, with the aim of improving their physicochemical and functional properties (Melnychyn, 1969; Alli & Baker, 1980). Previous work in our laboratory has shown that protein isolates prepared from *P. vulgaris*, with a crystalline microstructure, exhibited characteristics such as solubility, surface activity, and trypsin inhibitory activity that are different from protein isolates with an amorphous microstructure from the same beans (Li, Alli & Kermasha, 1989; Dilollo, Alli, Biliarderis & Barthakur, 1993). It has been shown that phaseolin polypeptides are the major components of the crystalline isolates of *P. vulgaris* (Alli, Gibbs, Okoniewska, Konishi & Dumas, 1993) and that, *P. lunatus* contain glycosylated and non-glycosylated variants that were similar to the C- and N-terminal segments of phaseolin polypeptides of *P. lunatus* (Alli, Gibbs, Okoniewska, Konishi & Dumas, 1994). The present study was carried out to investigate the differences between crystalline and amorphous isolates, with respect to the molecular structure of their constituent proteins, with reference to phaseolin, and the changes in primary structure that accompany the tryptic hydrolysis of protein isolates of *Phaseolus* beans.

2. Materials and methods

2.1. Materials

Samples of dried white kidney beans and navy beans (*Phaseolus vulgaris*), and large lima and baby lima beans (*Phaseolus lunatus*) were ground in a micro sample mill to pass through a 1 μm screen (Pulverising Machinery Ltd, Summit, NJ, USA) and stored in air-tight plastic containers at room temperature until they were used. Purified bovine milk casein and bovine pancreatic trypsin (93%) were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Preparation of crystalline and amorphous isolates

Protein isolates with a bipyramidal crystalline microstructure were prepared from *Phaseolus* beans according to the procedure of Melnychyn (1969). The ground beans (100 g) were extracted with citric acid solutions (1 litre). The bean flour suspensions were allowed to stand for 1 h at room temperature with intermittent stirring, then centrifuged (4000***g***, 10 min). The supernatant was filtered through glass wool and stored at 4°C for 18 h. The microstructures of the precipitated proteins were observed under a light microscope. The protein isolates were recovered by centrifugation (4000***g***, 10 min). The residues were washed with distilled, deionized water and lyophilized. Protein isolates with an amorphous microstructure were prepared from *Phaseolus* beans according to the method of Fan and Sosulski (1974). The ground beans (100 g) were extracted with dilute sodium hydroxide solution (1 litre, 0.02%). The suspensions were allowed to stand for 1 h at room temperature with intermittent stirring, then centrifuged (2500***g***, 10 min). The supernatant was filtered through glass wool, and the pH was adjusted to 4.5 with HCl to precipitate the proteins. The proteins were recovered by centrifugation (4000***g***, 10 min), washed with distilled water and lyophilized. The isolates were observed under a light microscope to confirm their amorphous microstructure.

2.3. Determination of protein content

The nitrogen content of the protein isolates and the ground beans was determined in duplicate according to the automatic Kjeldahl method (AOAC, 1985), using the Labconco Rapid Still III Kjeldahl system (Labconco Co-op, Kansas City, MO, USA).

2.4. Tryptic hydrolysis

Solutions of protein isolate (16 mg protein nitrogen ml^{-1}; pH 8.0) were mixed with trypsin solutions (0.64 mg ml^{-1}; pH 8.0) to give a mixture of final protein concentration of 8 mg ml^{-1} and an enzyme to substrate ratio of 1:25. The mixture was incubated at 38°C in a reciprocal shaker water bath for 3 h. At intervals of 5, 15, 30, 45, 90, and 180 min, aliquots of the hydrolysate (10 ml) were taken for the measurement of the nitrogen solubility index (NSI) and reversed phase high performance liquid chromatography (RP-HPLC) analysis.

2.5. NSI

The NSI was determined as the percentage of protein nitrogen solubilized in 10% (w/v) trichloroacetic acid

(Deeslie & Cheryan, 1988). Aliquots (10 ml) of the hydrolysate were added to 10 ml of 20% (w/v) trichloroacetic acid solution at 4°C. The resulting suspension was centrifuged (5000***g***, 10 min) and the nitrogen content of the supernatant determined by the automated Kjeldahl method (AOAC, 1985). The NSI was calculated using the equation described by Deeslie and Cheryan (1988). The effects of the time of hydrolysis, the bean variety, and the microstructure of the protein isolate on the NSI were analysed statistically by analysis of covariance in a randomized complete block design as $\log_e$(time of hydrolysis) $\times$ 4 levels of bean variety $\times$ 2 levels of extraction treatment. The differences in variables were estimated as the differences in their least square means (LSMEANS), and the *t*-test was used to test the level of significance.

2.6. RP-HPLC separation of tryptic peptides

The hydrolysates were subjected to RP-HPLC for separation of the peptides. A quantity (10 ml) of the hydrolysate was taken at intervals and heated rapidly to boiling to terminate the enzyme reaction. The hydrolysate was allowed to cool to room temperature and 1 ml of it was filtered (0.1 mm millex membrane Millipore Waters, St. Louis, MO, USA) and 20 µl of the filtrate analysed by HPLC using an LKB dual gradient pump system equipped with a diode array UV detector. Separation was carried out using a reversed phase analytical column (C_{18} LKB Ultro-Pac 10 µm pore size, 4 $\times$ 250 mm; LKB-Produkter AB, S-161 Bromma, Sweden), fitted with a guard column (Brownlee 3 cm C_{18}; Chromatographic Specialties Inc., Brockville, Ontario, Canada). A gradient mobile phase system: solvent A (0.1% TFA in distilled deionized water) and solvent B (0.09% TFA in acetonitrile), with a flow rate of 1 ml min^{-1} (maximum pressure 100 bar) and a linear gradient of 10% B to 50% B in 60 min was used for separation. The eluate was monitored at 214 nm. Fractions which gave a detector response at 214 nm were collected, dried in a speed vac and subjected to positive ion electrospray MS.

2.7. MS

The molecular weights of tryptic peptides were determined by ion-spray MS using a triple quadrupole mass spectrometer (API III MS/MS system, SCIEX, Thornhill, Ontario, Canada) according to the method described by Feng, Konishi and Bell (1991). Samples were infused into the electrospray ion source (fused silica capillary of 100 mm i.d.) at a rate of 1 ml min^{-1} from a low pressure infusion pump (Model 22, Harvard Apparatus, South Natick, MA, USA). The partial sequence analysis of peptides was determined from the Y_n ion series (daughter ion series formed by the cleavage of the peptide bond along the polypeptide backbone, with charge retention at the C-terminal fragment; providing sequence information in the C- to N-terminal direction) generated by collision-induced dissociation MS.

2.8. Identification of phaseolin fragments

The molecular weights of the identified peptides were compared with those of tryptic fragments generated theoretically from the sequence of α- and β-phaseolin from white kidney bean. Since peptides having different amino acid sequences can have similar molecular weights and mass to charge ratios (m/z) for their protonated $(M + n\mathrm{H})^{+n}$ molecular ions, the sequence of the peptides identified as tryptic fragments of α- and β-phaseolin was verified by MS/MS partial sequence analysis (Covey, Huang & Henion, 1991).

3. Results and discussion

Fig. 1 shows the effect of hydrolysis on the NSI of the hydrolysates of crystalline and amorphous protein isolates. The NSI of the unhydrolysed isolates were used as blanks. The NSI reflects the solubility properties of the hydrolysis products derived from the bean protein isolates, and can be used as an indicator for some functional properties of the protein isolates (Li-Chan, Nakai & Wood, 1984). The NSI of tryptic hydrolysates of the crystalline isolates was significantly higher ($p \leq 0.001$) than that of the amorphous isolates. The difference in the NSI between the crystalline and amorphous isolates can be

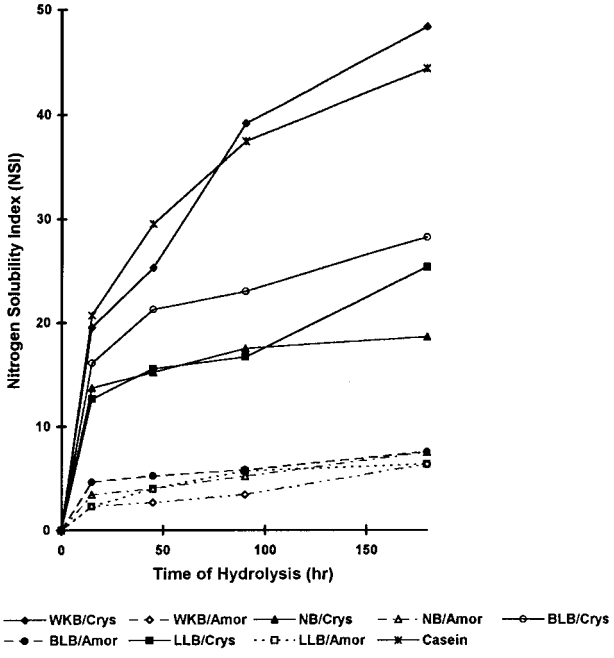

Fig. 1. Effect of time of hydrolysis on nitrogen solubility index of the protein isolates.

attributed to the conditions under which the crystalline isolates were prepared (cryoprecipitation), as opposed to the isoelectric precipitation method used to prepare the amorphous isolates. The higher NSI of the crystalline isolates compared with the amorphous isolates may also be due to a more extensive hydrolysis of the crystalline isolates.

Figs. 2 and 3 show the RP-HPLC chromatograms of the tryptic hydrolysates of the protein isolates from large lima bean and navy bean, respectively. The chromatograms at time 0 min represent the unhydrolysed protein isolates. Five minute hydrolysis resulted in several fractions in the crystalline isolates, which were not observed in the unhydrolysed (0 min) isolates. The chromatogram of the amorphous isolate after 5 min hydrolysis, on the other hand, did not show much difference from that of the unhydrolysed (0 min) amorphous isolate. These results suggests that the crystalline isolates were more susceptible to tryptic hydrolysis than the amorphous isolates. The peptide profile of the crystalline isolates did not change much after 5 min of hydrolysis, suggesting that the hydrolysis of the crystalline isolates was rapid and extensive during the first 5 min of hydrolysis. This result is in agreement with other studies on the proteolysis of phaseolin which suggest that the hydrolysis of phaseolin by digestive proteases, including trypsin, stops after only a small proportion of susceptible peptide bonds have been cleaved (Lienier & Thompson, 1980; Jivotovskaya et al., 1996). The results are also consistent with the results of the NSI (Fig. 1), which indicated a rapid and extensive hydrolysis in the first 5 min of hydrolysis. The differences between the RP-HPLC chromatograms of the crystalline and amorphous protein isolates is a clear indication of the structural differences of their constituent proteins in response to tryptic hydrolysis. Other factors which may account for the difference in the chromatograms of the crystalline and amorphous protein isolates include differences in the protein subunit structure (Li et al., 1989), and trypsin inhibitors. Previous studies have reported a higher level of trypsin inhibitory activity in the amorphous isolates than in the crystalline isolates (Li et al., 1989). The chromatograms of the crystalline and amorphous isolates of the other *Phaseolus* beans varieties (white kidney bean and baby lima bean) also demonstrated substantial differences between the two types of protein microstructures with respect to their response to tryptic hydrolysis (data not shown).

The HPLC fractions from the hydrolysates were collected and subjected to electrospray MS for the determination of molecular weight. The mass spectra of fraction 5 from the crystalline isolate of navy bean shows two mass ions at m/z 332.8 and 663.4, representing the $(M + 2H)^{+2}$ and $(M + H)^{+1}$ states of the same molecule (Fig. 4). The molecular weight of peptides, which are represented by two or more molecular ion states in a mass spectra, can be determined from the relationship described in Eq. (1). The molecular weight of a peptide represented by only one molecular ion in the mass spectra is, however, difficult to determine due

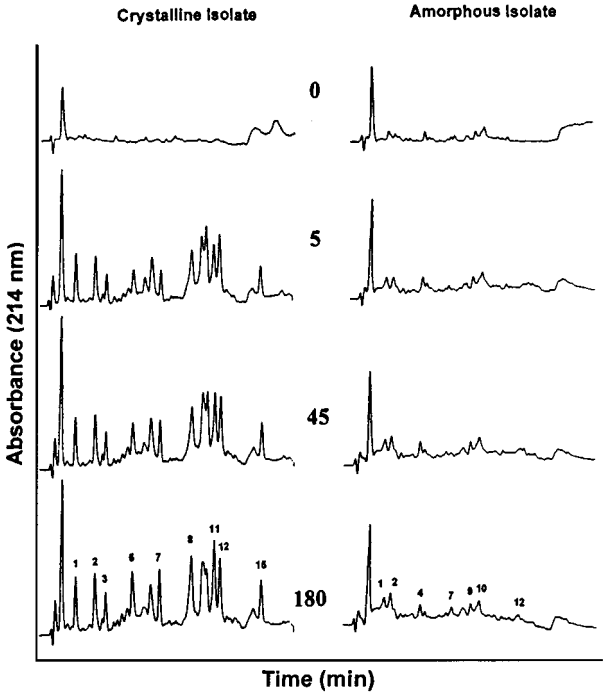

Fig. 2. Reverse phase-high performance liquid chromatography (RP-HPLC) chromatograms of the crystalline and amorphous isolates of large lima beans (LLB) at 0, 5, 45 and 180 min of tryptic hydrolysis.

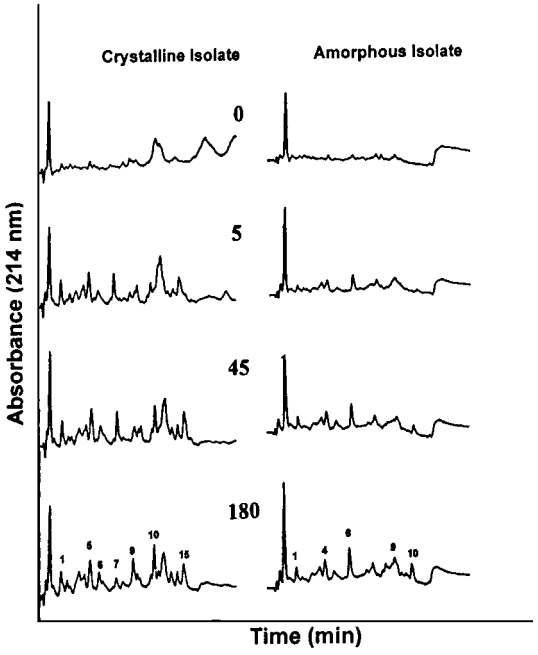

Fig. 3. Reverse phase-high performance liquid chromatography (RP-HPLC) chromatograms of the crystalline and amorphous isolates of navy bean (NB) at 0, 5, 45 and 180 min of tryptic hydrolysis.

to the uncertainty of assigning charge to the molecular ion. An examination of the peak profile of molecular ions in the mass spectra showed that singly charged molecular ions were associated with a series of isotope peaks that are separated by isotopic spacing of 1 Da (Fig. 5(A) insert), and doubly charged molecular ions showed isotopic spacing of 0.5 Da (Fig. 6(A) insert). These data show that the charged state of molecular ions can be determined by taking the reciprocal of the isotopic spacing. Charged states of molecular ions determined in this way were verified by collision-induced MS/MS. Singly charged parent ions generated daughter ions with lower mass (m) to charge (z) ratios than that of the parent ions, while molecular ions of higher charged state ($z > 1$), generated fragments or daughter ions with m/z values up to 'z' times the m/z value of the parent molecular ion (Fig. 5(B), where $z = 2$).

$$P_1 z_1 = \text{molecular weight of peptide } P + z_1(M_H) \quad (1)$$

where P_1 is the charge to mass ratio of peptide 'P', z_1 is the charge and M_H is the mass of a proton.

The determination of the charged state of a molecular ion using the isotopic spacing of its peak profile provides a means for the determination of the molecular weight of a peptide that is defined by only one molecular ion specie in a ESI mass spectra. This technique is useful, since the ESI MS of samples containing a mixture of peptides (e.g. RP-HPLC fractions of protein hydrolysates) usually produces a mixed mass spectra, containing a mixture of molecular ions.

Low molecular weight peptides (less than 1 kDa) showed abundant singly $(M + H)^+$ charged as well as doubly $(M + 2H)^{+2}$ charged molecular ions. As the molecular weight of peptides increased, the doubly charged molecular ion species $(M + 2H)^{+2}$ showed increasing dominance over the singly charged molecular ion species $(M + H)^+$. Tryptic peptides with a molecular weight > 1 kDa produced doubly charged molecular ion species $(M + 2H)^{+2}$ predominantly, and tryptic peptides with a molecular weight > 2 kDa produced intense triply charged molecular ion $(M + 3H)^{+3}$ species, as well as doubly charged species $(M + 2H)^{+2}$. The observed $(M + 3H)^{+3}$ ion may be due to the presence of an internal histidine residue in the peptide. The dominance of the $(M + 2H)^{+2}$ ion species over the $(M + 3H)^{+3}$ ion species can be attributed to the higher solution basicity of the N-terminal amino group and the C-terminal amino groups of lysine or arginine ($pK_a = 10$) compared with the ionizable nitrogen of histidine ($pK_a = 7$) (Covey et al., 1991).

Some tryptic peptide showed higher $(M + 3H)^{+3}$ abundance than the $(M + 2H)^{+2}$ molecular ion species.

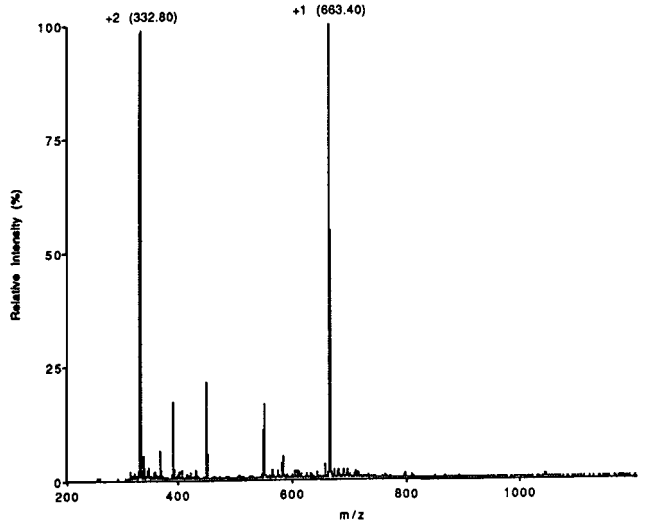

Fig. 4. Mass spectrum of fraction 5 of the tryptic hydrolysate of the crystalline isolate of navy bean, showing intense singly and double charged molecular ions of a peptide at m/z 663.4 and 332.8, respectively.

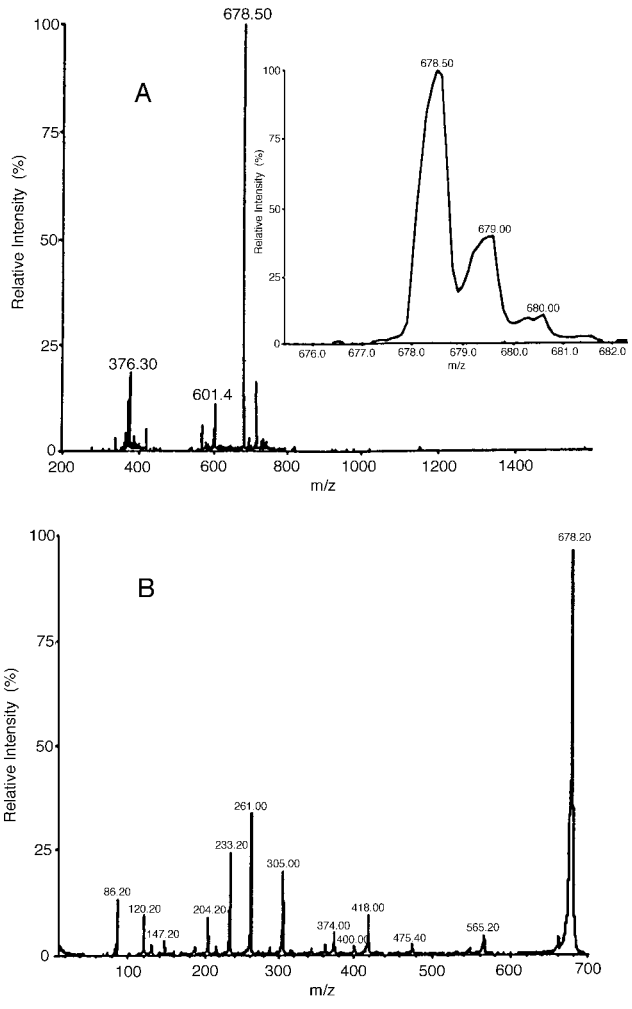

Fig. 5. (A) Mass spectrum showing a peak at m/z 678.5 and its isotopic spacing of 1 Da (insert). (B) The collision-induced dissociation (MSMS) daughter ion spectrum of the peak 678.5 in (A). The highest peak in the MSMS spectrum (678.2) has the same mass as the parent ion, showing that the peak in (A) is singly charged.

This situation could be due to the presence of internal lysine or arginine residues which are adjacent to a proline residue, and, therefore, resistant to tryptic hydrolysis. Other tryptic peptides produced abundant $(M + 3H)^{+3}$ and $(M + 4H)^{+4}$ molecular ions which were predominant over the $(M + 2H)^{+2}$ ion species. The molecular weights of peptides that showed ions of such high charged states were greater than 3 kDa. Some tryptic peptides showing intense $+3$ and $+4$ charged states that are predominant over the $+2$ charged state have been reported to be disulfide linked peptides (Covey et al., 1991). The molecular weight profile of the major peptide components of hydrolysates of both crystalline and amorphous protein isolates ranged from < 200 Da (i.e. amino acids) to > 5000 Da.

The collision-induced dissociation mass spectra of all the selected molecular ions were dominated by Y_n ion series (daughter ion series formed by the cleavage of the peptide bond along the polypeptide backbone, with charge retention at the C-terminal fragment; providing sequence information in the C- to N-terminal direction), suggesting that the C-terminal amino acid residues of the peptide fractions were basic amino acids, lysine and arginine.

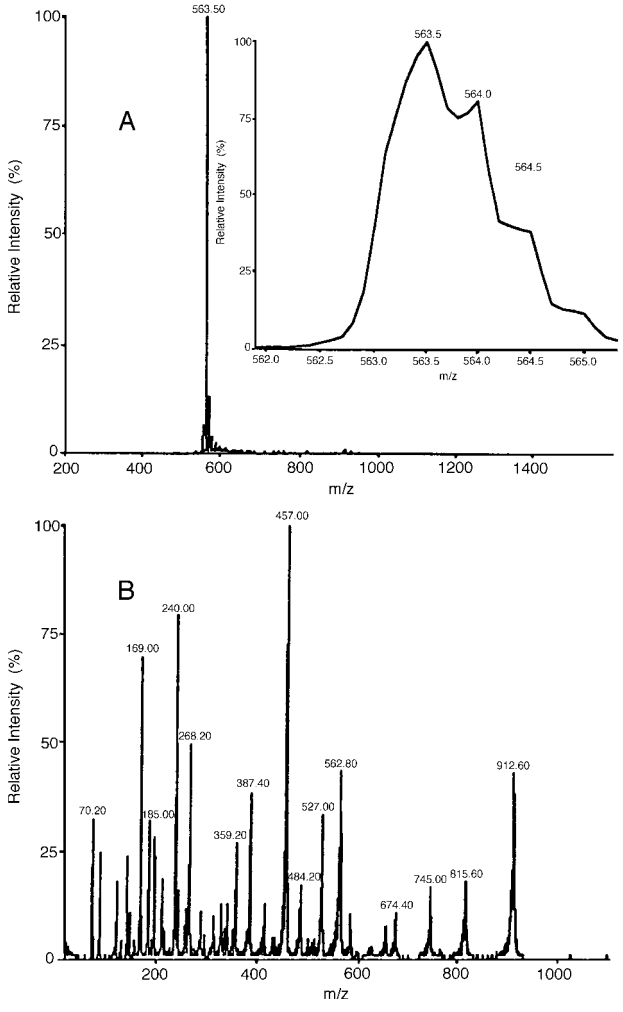

Fig. 6. (A) Mass spectrum showing a peak at m/z 563.5 and its isotopic spacing of 0.5 Da (insert). (B) The collision-induced dissociation (MSMS) daughter ion spectrum of the peak 563.5 in (A). The highest peak in the MSMS spectrum (912.6) is almost two times the mass of the parent ion, showing that the peak in (A) is doubly charged.

By comparing the sequence of the identified tryptic peptides from the hydrolysates of protein isolates from beans of *P. vulgaris* species (navy and white kidney beans) with theoretically generated tryptic fragments of α-phaseolin polypeptide, the origin of some of the identified peptides were traced to α- and β-phaseolin polypeptides. This association could not be done with the hydrolysates of the protein isolates of *P. lunatus* beans (baby and large lima beans), since the sequence of the phaseolin polypeptide from *P. lunatus* species is not known. Table 1(a) and (b) show the sequence of peptides identified as tryptic fragments of β-phaseolin from the hydrolysate of the crystalline and amorphous isolates of navy bean after 180 min of tryptic hydrolysis, and Fig. 7(a) and 7(b) represent the sequence of β-phaseolin (Slighton, Drong, Klassy & Hoffman, 1985) polypeptide showing the peptide bond that must been cleaved to produce the identified peptides in Table 1. β-Phaseolin polypeptide from the crystalline isolate is referred to as phaseolin-C, and β-phaseolin polypeptide from the amorphous isolate is referred to as phaseolin-A. Table 2(a–c) shows the structural regions from which the cleaved loci originate.

The results show that the cleaved peptide bonds are mainly located in regions interconnecting secondary structural elements (K_{68}–R_{69}, R_{69}–L_{70}, R_{77}–L_{78}, K_{283}–A_{284}, K_{303}–G_{304}, R_{315}–A_{316}, K_{320}–D_{321}, K_{360}–T_{361}, and R_{370}–A_{371}), and in α-helical structures (K_{187}–H_{188},

Table 1
Sequence of tryptic fragments of β-phaseolin identified in the hydrolysates of the crystalline and amorphous isolates of navy bean

Sequence	MH^+	β-Phaseolin
(a) Crystalline isolate		
R-**AELSK**-D	547.3	316–320
R-**KSLSK**-Q	562.3	237–241
R-**NLLAGK**-T	615.5	355–360
K-**FEEINR**-V	807.5	198–203
R-**LQNLEDYR**-L	1050.3	70–77
K-**TDNVISSIGR**-A	1061.3	361–370
K-**HILEASFNSK**-F	1145.3	188–197
K-**AIVILVVNEGEAHVELVGPK**-G	2086.3	284–303
R-**VLFEEEGQQEGVIVNIDSEQIK**-E	2503.7	204–225
(b) Amorphous isolate		
K-**GR**-K	232.3	414–415
R-**KSLSK**-Q	562.3	237–241
R-**NLLAGK**-T	615.5	355–360
R-**LQNLEDYR**-L	1050.3	70–77
K-**TDNVISSIGR**-A	1061.3	361–370
K-**HILEASFNSK**-F	1145.3	188–197
K-**RLQNLEDYR**-L	1206.8	69–77

Fig. 7. (a) Sequence of β-phaseolin (phaseolin-C) showing the possible cleavage loci according to the tryptic peptides identified from the hydrolysates of the crystalline isolate of navy beans (see Table 1(a)). Arrows show the cleavage points. (b) Sequence of β-phaseolin (phaseolin-A) showing the possible cleavage loci according to the tryptic peptides identified from the hydrolysates of the amorphous isolate of navy beans (see Table 1(b)). Arrows show the cleavage points.

Table 2
Location of cleaved peptide bonds of β-phaseolin in the hydrolysates of the protein isolates of navy bean (Table 1); locations are based on the model of Lawrence et al. (1994)

(a) Trypsin susceptible peptide bond cleaved in the N-terminal structural domain	
K_{68}–R_{69}	Connecting secondary structural units
R_{69}–L_{70}	Connecting secondary structural units
R_{77}–L_{78}	Connecting secondary structural units
K_{187}–H_{188}	Forms part of the second α-helical structure
K_{197}–F_{198}	Forms part of the third α-helical structure
R_{203}–V_{204}	Forms part of the third α-helical structure
K_{225}–E_{226}	Forms part of the fourth α-helical structure
(b) Trypsin susceptible peptide bonds located in the inter-domain linker	
R_{236}–K_{237}	Disordered region of the inter-domain linker
K_{241}–Q_{242}	Disordered region of the inter-domain linker
(c) Trypsin susceptible peptide bond cleaved in the C-terminal structural domain	
K_{283}–A_{284}	Connecting secondary structural units
K_{303}–G_{304}	Connecting secondary structural units
R_{315}–A_{316}	Connecting secondary structural units
K_{320}–D_{321}	Connecting secondary structural units
R_{354}–N_{355}	Connecting secondary structural units
K_{360}–T_{361}	Connecting secondary structural units
R_{370}–A_{371}	Connecting secondary structural units
K_{413}–G_{414}	C-terminal
R_{415}–K_{416}	C-terminal

K_{197}–F_{198}, R_{203}–V_{204}, K_{225}–E_{226}). The structure of β-phaseolin used for this assessment is based on the high resolution X-ray structural model of Lawrence et al. (1994). The model shows that the β-phaseolin subunit consists of two structurally similar domains (C-terminal and N-terminal domains). Each domain is made up of two structural elements: a compact eight stranded β-barrel structure and an extended loop consisting of three α-helixes. The two domains are connected by a sequence consisting of a helical structure and a random coil. Two bonds, R_{236}–K_{237}, and K_{241}–Q_{242} located in a disordered section of the interdomain linker have been previously identified as the most susceptible peptide bonds to tryptic hydrolysis (Jivotovskaya et al., 1996). Cleavage of these bonds divides the phaseolin polypeptide into two structurally identical halves. The trypsin susceptible peptide bonds that were not cleaved are located in regions rich in hydrophobic residues (M_1–K_{68}), or proline residues (S_{104}–K_{151}). These regions also form part of a compact β-barrel structure (Lawrence et al., 1994; Jivotovskaya et al., 1996) which does not seem to be accessible for tryptic hydrolysis.

Fewer tryptic peptides of β-phaseolin were identified in the hydrolysate of the amorphous isolate when compared with that of the crystalline isolate (Table 1(a) and (b)). This suggests that the structure of phaseolin in the amorphous isolate (phaseolin-A) is more compact, and more resistant to tryptic hydrolysis than phaseolin in the crystalline isolate (phaseolin-C). Fig. 7(a) and (b) also reveals some subtle structural differences between phaseolin-C and phaseolin-A. Although phaseolin-C was hydrolysed in more places than phaseolin-A, phaseolin-A showed cleavage of K_{413}–G_{414} and R_{415}–K_{416} peptide bonds in the C-terminal end of the polypeptide, as well as K_{68}–R_{69} peptide bond in the N-terminal region, which were not observed for phaseolin-C.

4. Conclusion

The marked difference between the hydrolytic products of the crystalline and amorphous protein isolates with respect to their solubility properties indicates that crystalline isolates may have a high potential as functional ingredients in the food industry. The RP-HPLC chromatograms of the hydrolysates of the protein isolates also confirm previous studies which have reported that phaseolin (the major component of *Phaseolus* sp.) is particularly resistant to tryptic hydrolysis, and that its hydrolysis stops after only a few peptide bonds have been cleaved.

The tryptic peptides identified in the hydrolysates indicate cleavage loci of β-phaseolin by trypsin that are structurally consistent with the X-ray crystallographic model of phaseolin reported by Lawrence et al. (1994). The results show that the trypsin-specific peptide bonds (peptide bonds with arginine or lysine at the N-terminal

end of the bond) that were cleaved are located in the most structurally accessible regions of the polypeptide, while those that were not cleaved are located in the most compact and structurally constrained β-structured regions of the protein. The results also show the existence of subtle structural differences between phaseolin-C and phaseolin-A.

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