# Tryptic Hydrolysis (in Vitro) of Crystalline and Noncrystalline Proteins from *Phaseolus* Beans

Zhuo Li, Inteaz Alli,* and Selim Kermasha

Crystalline protein isolates and amorphous protein isolates were prepared from the following four types of beans: white kidney, navy (*Phaseolus vulgaris*), baby lima, large lima (*Phaseolus lunatus*). The degree of tryptic hydrolysis (in vitro) and the tryptic inhibitory (TI) activity of the two types of microstructures were measured. The crystalline isolates showed lower TI activity than the amorphous isolates. However, the extent of tryptic hydrolysis was not related to the TI activity or to the phytate content of the isolates. Sodium dodecyl sulfate–polyacrylamide disc gel electrophoresis indicated that the major protein fractions of the *P. lunatus* beans were more resistant to tryptic hydrolysis than the major fractions of the *P. vulgaris* beans.

Evaluation of plant proteins in general, and legume seed proteins in particular, for utilization in foods often includes investigation of the biological properties of the proteins. The occurrence of protease inhibitory activity in some legume seeds is a matter of concern. The effect of trypsin inhibitors on pancreatic function and consequently on the overall proteolytic digestive process has been documented (Rackis et al., 1985). Trypsin inhibitors from various legume seeds have been isolated, purified, and characterized (Weder, 1985; Whitaker and Sgarbieri, 1981).

Trypsin inhibitors are not the only factors shown to inhibit tryptic hydrolysis of proteins (Kakade, 1974). The inhibition of trypsin by phytate (Singh and Krikorian, 1982) and the involvement of phenolic compounds in trypsin inhibitory activity (Bressani et al., 1983; Fernandez et al., 1982; Griffith and Moseley, 1980) have been reported. The low digestibility of some legume seed proteins has also been attributed to the compact structure of these storage proteins (Chang and Satterlee, 1981; Leavitt et al., 1977). Kakade (1974) discussed the influence of primary and tertiary structure of proteins in the rate of their hydrolysis. The reduction of protein digestibility as a result of storage of legume seeds has also been reported (Eggum and Beames, 1983).

In our studies on the isolation of proteins from beans (*Phaseolus*), we have investigated the properties of proteins exhibiting crystalline and noncrystalline microstructures. Previous reports from these studies have dealt with different types of microstructures of the isolated proteins (Alli and Baker, 1980), the nature of protein–phytate interactions (Alli and Baker, 1981), and the electrophoretic and chromatographic behavior of the proteins (Alli and Baker, 1983; Musakhanian and Alli, 1987). The present work was carried out to evaluate the degree of (in vitro) tryptic hydrolysis of proteins exhibiting crystalline and noncrystalline microstructures and to identify the factors that markedly affect the extent of tryptic hydrolysis of the proteins.

## MATERIALS AND METHODS

**Materials.** Proteins exhibiting bipyramidal crystalline microstructures were extracted from white kidney beans and navy beans (*Phaseolus vulgaris*) and from baby lima beans and large lima beans (*Phaseolus lunatus*). The proteins were extracted with citric acid solution (Table I) and crystallized by storing under refrigeration (Melnychyn, 1969). The concentration and pH of the citrate extracting solutions were established by referring to previous work (Alli, 1977), which indicated the likelihood of obtaining crystalline protein. Proteins exhibiting amorphous microstructures were prepared from the same beans by extraction with sodium hydroxide solution (0.02%) followed by isoelectric precipitation (Fan and Sosulski, 1974). The proteins were recovered from the extracts by centrifugation and lyophilized. Figure 1 illustrates the microstructures of the two types of proteins.

**Table I. Conditions Used for Extraction of Crystalline Proteins from *Phaseolus* Beans**

| bean | concn, N/pH of citrate extr soln | bean | concn, N/pH of citrate extr soln |
|---|---|---|---|
| white kidney | 0.08/5.5 | baby lima | 0.10/3.0 |
| navy | 0.20/5.0 | large lima | 0.10/3.0 |

**Tryptic Hydrolysis of Proteins.** The extent of tryptic hydrolysis of the crystalline and amorphous proteins was determined according to the formol titration "direct" method of Taylor (1957). A quantity of lyophilized protein isolate equivalent to 200 mg of pure protein was suspended in distilled water (40 mL). The suspension was adjusted to pH 8.0 by dropwise addition of 10% NaOH and the volume adjusted to 50 mL with distilled water. Trypsin solutions were prepared by dissolving 250 mg of trypsin (hog pancreas; protease activity 300 USP units/mg of powder; ICN Biomedicals, Inc.) in distilled water (40 mL), adjusting to pH 8.0, and then diluting to 50 mL with distilled water. The protein suspension and the trypsin solution were brought to 38 °C separately and then mixed. Immediately after mixing, 10 mL of the mixture was taken, heated to stop the reaction, and then analyzed for $\alpha$-amino nitrogen by the formol titration direct method. This digestion mixture at zero time was used as the blank (Taylor, 1957). Additional 10-mL quantities were taken after 20, 40, 60, 90, 120, 150, and 180 min and analyzed for $\alpha$-amino nitrogen. Tryptic hydrolysis is expressed as milligrams of $\alpha$-amino nitrogen liberated from 1 g of protein.

**Trypsin Inhibitory Activity.** Trypsin inhibitory activity was determined by the benzoyl-DL-arginine *p*-nitroanilide (BAPA) substrate procedure of Kakade et al. (1969). A quantity of lyophilized protein isolate, equivalent to 100 mg of pure protein, was suspended in 50 mL of Tris-HCl buffer (50 mM, pH 8.2, containing 70 mM $CaCl_2$), agitated intermitently for 1 h, and then centrifuged. Quantities (0, 0.2, 0.4, 0.6, 0.8, 1 mL) of the supernatant were placed in separate test tubes, and the volume was adjusted to 1 mL with distilled water. Trypsin solution (1 mL) prepared by dissolving 250 mg of trypsin powder (hog pancreas; 300 USP units/mg of powder; ICN Biomedicals) in 100 mL of 0.001 N HCl was added to each test tube. The tubes were placed in a water bath at 37 °C. To each tube was added 7 mL of BAPA solution, which was previously warmed to 37 °C. Exactly 10 min after addition of the BAPA solution, acetic acid (1 mL, 30%) was added to stop the reaction. The solution was mixed and the absorbance measured at 410 nm. Trypsin inhibitory activity is expressed as

Department of Food Science and Agricultural Chemistry, Macdonald College of McGill University, 21,111 Lakeshore Road, Ste. Anne de Bellevue, Québec, Canada H9X 1C0.

0021-8561/89/1437-0643$01.50/0 

**Table II. Tryptic Hydrolysis of Proteins of *Phaseolus* Beans and Casein**

| | mg α-amino nitrogen liberated from 1 g bean protein[a] | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | white kidney | | navy | | baby lima | | large lima | | |
| hydrolysis time, min | c | a | c | a | c | a | c | a | casein |
| 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 20 | 7.33 | 7.61 | 11.03 | 11.03 | 8.32 | 11.24 | 0.94 | 5.34 | 6.76 |
| 40 | 16.6 | 12.4 | 12.6 | 19.8 | 11.2 | 15.9 | 2.13 | 11.6 | 14.9 |
| 60 | 20.1 | 20.1 | 14.2 | 26.1 | 12.4 | 17.1 | 2.70 | 12.4 | 22.8 |
| 90 | 28.2 | 20.6 | 14.5 | 26.3 | 16.2 | 24.9 | 7.83 | 21.7 | 47.7 |
| 120 | 32.3 | 26.9 | 14.5 | 27.0 | 16.9 | 29.2 | 12.1 | 22.8 | 64.0 |
| 150 | 44.3 | 29.7 | 22.7 | 32.0 | 16.9 | 36.3 | | | |
| 180 | 70.7 | 48.5 | 45.28 | 46.3 | 25.4 | 45.1 | 20.1 | 32.4 | 87.7 |

[a] Key: c, crystalline; a, amorphous.

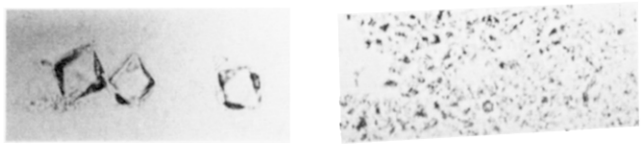

**Figure 1.** Microscopic structure (light microscope) of crystalline and amorphous protein isolates of white kidney beans (magnification ×100).

trypsin units inhibited (TUI), which is defined as the increase in absorbance units (at 410 nm) per 10-min reaction time under the conditions of the experiment (Kakade et al., 1969).

**Phytate Content.** The phytate content of the proteins was determined by the method of Haug and Lantzsch (1983), with some modifications. Protein (100 mg) was extracted with HCl solution (10 mL, 0.2 N). The mixture was filtered, and 2.5 mL of the filtrate was diluted to 10 mL. A quantity (0.5 mL) of the filtrate was mixed with HCl solution (10 mL, 0.2 N) containing ferric ammonium sulfate (0.5 mM) and two drops of saturated bromine water. The mixture was heated (30 min, boiling water bath), cooled (15 min, ice bath), and then centrifuged (30 min, 5000*g*) to precipitate any ferric phytate formed during the reaction. The iron content of the supernatent was determined by adding 2,2′-bipyridyl/mercaptoacetic acid solution (1.5 mL) to the supernatant (1 mL) followed by colorimetric analysis at 519 nm. A standard curve was prepared with use of sodium phytate (Sigma Chemical Co.).

**SDS–Polyacrylamide Disc Gel Electrophoresis.** Sodium dodecyl sulfate–polyacrylamide disc gel electrophoresis (SDS-PAGE) was carried out with the procedure of Weber et al. (1972). The proteins subjected to tryptic hydrolysis and the hydrolysates obtained after 3-h digestion were analyzed; the hydrolysates (5 mL) were dialyzed against distilled water before they were subjected to electrophoresis.

## RESULTS AND DISCUSSION

The amounts of α-amino acids liberated from the isolated bean proteins were lower than that of casein after 90 min of tryptic hydrolysis (Table II). This could be due to one or more of the following factors: the presence of trypsin inhibitors (Rackis et al., 1985), phytate (Singh and Krikorian, 1982), polyphenolic compounds (Bressani et al., 1983) in the bean proteins but not in casein, and/or the more ordered and helical structure of the legume seed proteins as compared with casein (Bhatty, 1988). Casein contained no phytate and relatively low levels of trypsin inhibitor activity (Table III). Three of the four amorphous isolates of the beans (the amorphous protein isolate from the navy, baby lima, and large lima beans) showed greater hydrolysis than the bipyramidal crystalline isolate of the same beans. On the other hand, the crystalline isolate from the white kidney bean showed a greater extent of hydrolysis than the amorphous isolate. This suggests that there is no clear relationship between the tryptic hydrolysis and microstructures of the protein isolates.

In all instances, the amorphous protein isolates showed considerably higher levels of TI activity when compared

**Table III. Trypsin Inhibitory Activity of Proteins of *Phaseolus* Beans and Casein**

| | trypsin units inhibited (TUI)/1 mg protein | | |
|---|---|---|---|
| bean | crystalline | amorphous | casein |
| white kidney | 2.6 | 57.5 | 0.5 |
| navy | 1.2 | 29.1 | |
| baby lima | 13.6 | 104.7 | |
| large lima | 38.7 | 78.5 | |

**Table IV. Phytate Content of Proteins from *Phaseolus* Beans[a]**

| | g phytic acid/100 g protein | |
|---|---|---|
| bean proteins | crystalline | amorphous |
| white kidney | 2.37 | 6.62 |
| navy | 3.88 | 7.59 |
| baby lima | 5.75 | 5.08 |
| large lima | 7.68 | 5.25 |

[a] Results are means of triplicate analyses.

to the crystalline protein isolates (Table III), suggesting a relationship between the microstructure of the proteins and their TI activity. The TI activities of the two *P. vulgaris* beans (white kidney, navy) were much lower than those of the two *P. lunatus* beans (baby lima, large lima), suggesting that the differences in TI activity might also be related to species variation. The crystalline protein isolates contained lower levels of trypsin inhibitors than the amorphous protein isolates from the same bean. The differences in TI activity between the crystalline and amorphous isolates do not correlate with the differences in observed tryptic hydrolysis (Table II). On the one hand, the amorphous isolates of navy, baby lima, and large lima beans showed higher tryptic hydrolysis than the crystalline isolates of the same bean. But the TI activities of the crystalline isolates were considerably lower than those of the amorphous isolates. This suggests that the TI activity of the bean proteins alone does not account for their degree of tryptic hydrolysis. Jaffé (1950) suggested that there appeared to be no correlation between the activity of trypsin and protein digestibility. Nevertheless, the interference of TI in pancreatic function is well documented (Liener, 1979). Other workers (Thompson et al., 1986; Singh and Krikorian, 1982; Fernandez et al., 1982; Chang and Statterlee, 1981; Romero and Ryan 1978) have implicated various other factors in their studies on enzymatic hydrolysis of bean proteins.

There was no relation between the phytate content (Table IV) of the proteins (crystalline amorphous) and the extent of tryptic hydrolysis. For instance, with white kidney bean, the extent of hydrolysis of the crystalline isolate (2.37% phytate) was higher than that of the amorphous isolate (6.62% phytate). On the other hand, with the navy bean, the extent of hydrolysis of the crys-

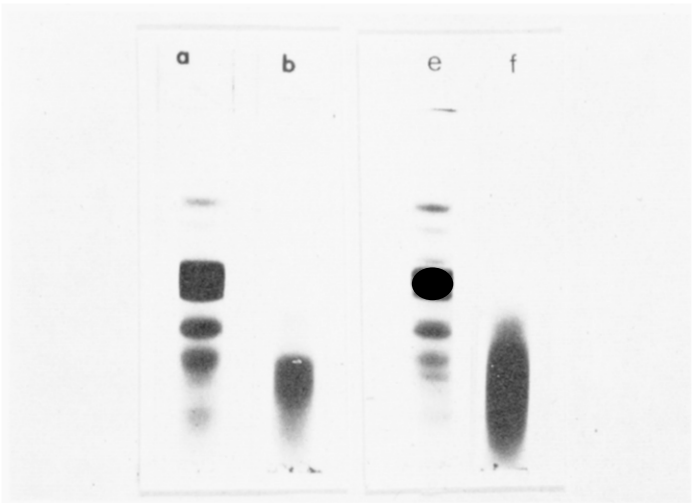


**Figure 2.** Electropherograms of unhydrolyzed and hydrolyzed proteins of white kidney beans: (a) unhydrolyzed crystalline protein; (b) hydrolyzed crystalline protein; (e) unhydrolyzed amorphous protein; (f) hydrolyzed amorphous protein. Electrophoresis performed in the presence of SDS with a sodium phosphate (0.2 M, pH 7.2) buffer system (Weber et al., 1972).

talline isolate (3.85% phytate) was lower than that of the amorphous isolate (7.59% phytate). Previous work has shown that added phytate inhibited the tryptic digestion (Singh and Krikorian, 1982) and the peptic digestion (Knuckles et al., 1985) of casein.

Figure 2 shows the electropherograms of the crystalline protein (a), the hydrolyzed crystalline protein (b), the amorphous protein (e), and the hydrolyzed amorphous protein (f) of the white kidney beans. The electrophoretic behavior of the unhydrolyzed crystalline and amorphous proteins (a, e) was similar; this supports findings reported previously (Alli, 1979). The major protein fraction of both the crystalline and amorphous protein was completely hydrolyzed by trypsin. In addition, several of the relatively minor protein fractions were also completely hydrolyzed. The protein material remaining after 180-min hydrolysis appeared as an unresolved diffused band in both the crystalline isolate and amorphous isolate. Qualitatively, the more intense staining in the hydrolyzed amorphous protein suggests higher quantities of protein material as compared with hydrolyzed crystalline isolate. This agrees with the results of liberation of free $\alpha$-amino acids (Table II), which showed that the crystalline protein was hydrolyzed to a greater extent than the amorphous protein.

Figure 3 shows the electropherograms obtained with the proteins from the navy beans. The electrophoretic behavior of the unhydrolyzed proteins of the navy bean was similar to that of the white kidney bean; this supports the findings obtained previously (Alli, 1979). This could be related to the fact that these two beans are varieties of the same species (*P. vulgaris*). As was observed with the white kidney bean proteins, the major fraction of the navy bean proteins, along with several of the minor fractions, were completely hydrolyzed during the 180-min hydrolysis period. Unlike the white kidney bean proteins, the electropherograms of the hydrolyzed navy bean proteins (h, j) showed distinct fractions with relatively high migration; these fractions could represent low molecular peptides produced during the incomplete hydrolysis of the proteins. Romero and Ryan (1978) reported that SDS electrophoresis of a major protein fraction of *P. vulgaris* bean confirmed the susceptibility of the protein to tryptic hydrolysis.

Figure 4 shows the electropherograms of the protein isolates from the baby lima beans. The observation that

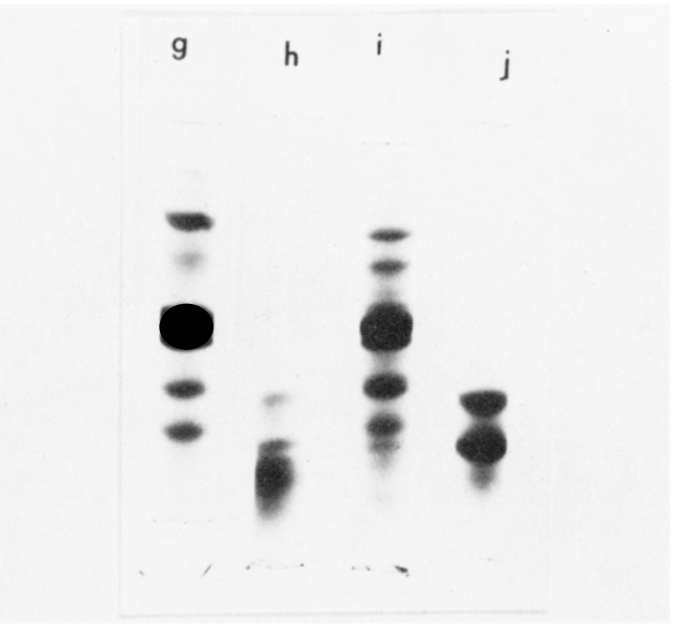


**Figure 3.** Electropherograms of unhydrolyzed and hydrolyzed proteins of navy beans: (g) unhydrolyzed crystalline protein; (h) hydrolyzed crystalline protein; (i) unhydrolyzed amorphous protein; (j) hydrolyzed amorphous protein. Electrophoresis performed in the presence of SDS with a sodium phosphate (0.2 M, pH 7.2) buffer system (Weber et al., 1972).

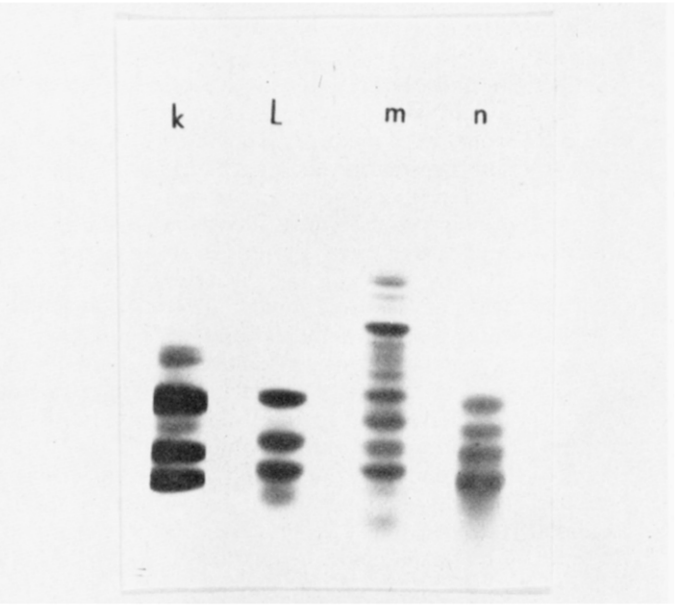


**Figure 4.** Electropherograms of unhydrolyzed and hydrolyzed proteins of baby lima beans: (k) unhydrolyzed crystalline protein; (l) hydrolyzed crystalline protein; (m) unhydrolyzed amorphous protein; (n) hydrolyzed amorphous protein. Electrophoresis performed in the presence of SDS with a sodium phosphate (0.2 M, pH 7.2) buffer system (Weber et al., 1972).

the electropherograms of the unhydrolyzed crystalline isolate (k) was markedly different from that of the unhydrolyzed amorphous isolate (m) is similar to that reported previously (Alli and Baker, 1983). This suggests a difference in the molecular weight distribution of the crystalline and amorphous proteins. The unhydrolyzed crystalline isolate (k) was characterized by the presence of three major fractions and relatively few minor fractions; the amorphous isolate (m) was characterized by several major as well as several minor fractions. The higher molecular weight minor fractions in the amorphous isolate were not present in the crystalline isolate. The elec-

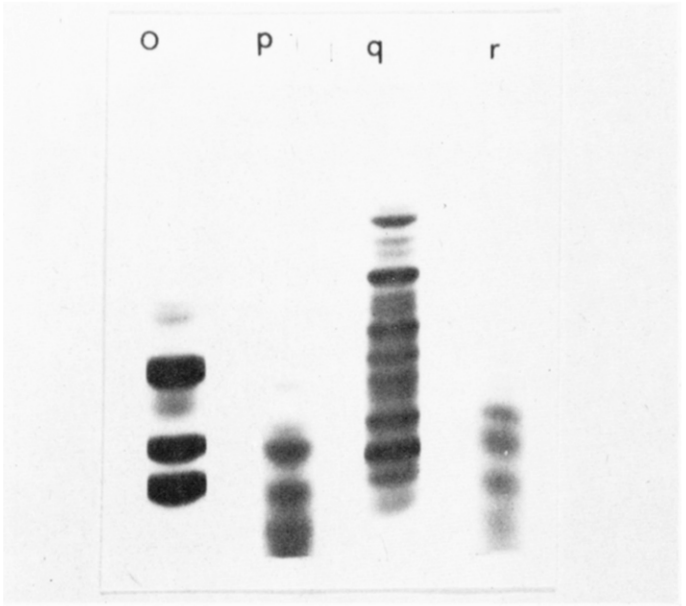


**Figure 5.** Electropherograms of unhydrolyzed and hydrolyzed proteins of baby lima beans: (o) unhydrolyzed crystalline protein; (p) hydrolyzed crystalline protein; (a) unhydrolyzed amorphous protein; (r) hydrolyzed amorphous protein. Electrophoresis performed in the presence of SDS with a sodium phosphate (0.2 M, pH 7.2) buffer system (Weber et al. 1972).

tropherograms of the hydrolyzed crystalline isolate (l) indicate that the three major protein fractions were somewhat resistant to tryptic hydrolysis. The electropherograms of the hydrolyzed amorphous isolate (n) indicate that the fractions with lower migration (higher molecular weight fractions) were readily hydrolyzed by trypsin. The major fractions present in the amorphous isolate (like the major fractions in the crystalline proteins) were relatively resistant to hydrolysis. The results from the large lima bean protein (Figure 5) were similar to those of the baby lima bean proteins; this indicates intraspecific similarities.

On the bases of qualitative evaluation of the electrophoresis results, there is an indication that there is greater resistance to proteolysis by proteins from *P. lunatus* beans when compared with the *P. vulgaris* beans; this can be correlated to the higher trypsin inhibitory activity of the proteins of *P. lunatus* beans (Table III). Eggum and Beames (1983) reported that there is a large variation in protein digestibility both within and between species. The results also suggest that the trypsin inhibitory activity of the *P. lunatus* beans might be located in the major fractions.

## CONCLUSION

The present study has shown that the trypsin inhibitory activities of bean proteins with crystalline microstructure (extracted with citric acid solution) are lower than that of bean proteins with amorphous microstructures (extracted with dilute alkali solution). However, there was no direct relationship between the trypsin inhibitory activity and the extent of tryptic hydrolysis of the proteins. In addition, no relationship was found between the tryptic hydrolysis and the phytate content of the protein. SDS-PAGE demonstrated that certain major protein fractions of *P. lunatus* beans were more resistant to trypsin hydrolysis when compared with *P. vulgaris* beans.

## ACKNOWLEDGMENT

This research was supported by an operating grant from the Natural Sciences and Engineering Research Council of Canada.

## LITERATURE CITED

Alli, I. Isolation and characterization of proteins from *Phaseolus* beans. M.Sc. Thesis, McGill University, 1977.

Alli, I. Isolation and characterization of crystalline and noncrystalline proteins from *Phaseolus* beans. Ph.D. Thesis, McGill Univeristy, 1979.

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

Alli, I.; Baker, B. E. Constitution of leguminous seeds. A note on protein-phytic acid interactions during isolation of acid-soluble protein from *Phaseolus* beans. *J. Sci. Food Agric.* **1981**, *32*, 588–592.

Alli, I.; Baker, B. E. Constitution of legumonous seeds: Polyacrylamide disc gel electrophoresis of acid soluble and acid precipitated proteins prepared from baby lima beans. *J. Sci. Food Agric.* **1983**, *34*, 737–742.

Bhatty, R. S. In vitro hydrolysis of pea, faba bean and lentil meals and isolated protein fractions by pepsin and trypsin. *Can. Inst. Food Sci. Technol. J.* **1988**, *21*, 66–71.

Bressani, R.; Elias, L. G.; Wolzak, A.; Hagerman, A. E.; Butler, L. G. Tannin in common beans: methods of analysis and effects on protein quality. *J. Food Sci.* **1983**, *48*, 1000–1001, 1003.

Chang, K. C.; Satterlee, L. D. Isolation and characterization of the major protein from Great Northern beans (*Phaseolus vulgaris*). *J. Food Sci.* **1981**, *46*, 1368–1373.

Eggum, B. O.; Beames, R. M. The nutritive value of seed proteins. In *Seed Proteins: Biochemistry, Genetics, Nutritive Value*; Gottschalk, W., Müller, H. P., Eds.; Martin Nijhoff/Dr. W. Junk Publishers: The Hague, The Netherlands, 1983; pp 497–531.

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

Fernandez, R.; Elias, L. G.; Braham, J. E.; Bressani, R. Trypsin inhibitors and hemagglutinins in beans (*Phaseolus vulgaris*) and their relationship with the content of tannins and associated polyphenols. *J. Agric. Food Chem.* **1982**, *30*, 734–739.

Griffith, D. W.; Moseley, G. The effects of diets containing field-beans of high and low phenolic content on the activity of digestive enzymes in the intestine of rats. *J. Sci. Food Agric.* **1980**, *31*, 255–259.

Haug, W.; Lantzsch, H. J. Sensitive method for the determination of phytate in cereals and cereal products. *J. Sci. Food Agric.* **1983**, *34*, 1423–1426.

Jaffé, W. G. Protein digestibility and trypsin inhibitor activity of legume seeds. *Proc. Soc. Exp. Biol. Med.* **1950**, *75*, 219–220.

Kakade, M. L. Biochemical basis for the differences in plant protein utilization. *J. Agric. Food Chem.* **1974**, *22*, 550–555.

Kakade, M. L.; Simons, N.; Liener, I. E. An evaluation of natural vs synthetic substrates for measuring the antitryptic activity of soybean samples. *Cereal Chem.* **1969**, *46*, 518–521.

Knuckles, B. E.; Kuzmicky, D. D.; Betschart, A. A. Effect of phytate and partially hydrolyzed phytate on vitro protein digestion. *J. Food Sci.* **1985**, *50*, 1080–1082.

Leavitt, R. D.; Felsted, R. D.; Bachur, N. R. Biological and biochemical properties of *Phaseolus vulgaris* isolectins. *J. Biol. Chem.* **1977**, *252*, 2961–2966.

Liener, I. E. The nutritional significance of plant protease inhibitors. *Proc. Nutr. Soc.* **1979**, *38*, 109–113.

Melnychyn, P. Process for isolating proteins from beans in a crystalline state using an alkaline salt of carboxylic acid. U.S. Patent 3,450,688, 1969.

Musakhanian, J.; Alli, I. Fractionation by gel exclusion HPLC of proteins from acidic and alkaline extraction of *Phaseolus* beans. *Food Chem.* **1987**, *23*, 223–234.

Rackis, J. J.; Gumbmann, M. R.; Liener, L. E. The USDA trypsin inhibitor study. I. Background, objectives, and procedural details. *Qual. Plant Foods Hum. Nutr.* **1985**, *35*, 213–242.

Romero, J.; Ryan, D. S. Susceptibility of the major storage protein of the bean, *Phaseolus vulgaris* L., to in vitro enzymatic hydrolysis. *J. Agric. Food Chem.* **1978**, *26*, 784–788.

Singh, M.; Krikorian, A. D. Inhibition of trypsin activity in vitro by phytate. *J. Agric. Food Chem.* **1982**, *30*, 799–800.

Taylor, W. H. Formal titration: An evaluation of its various modifications. *The Analyst* **1957**, *82*, 488–497.

Thompson, L. U.; Tenebaum, A. V.; Hui, H. Effect of lectins and mixing of proteins on rate of protein digestion. *J. Food Sci.* **1986**, *51*, 150–152, 160.
Weber, K.; Pringle, J. R.; Osborn, M. Measurement of molecular weights by electrophoresis on SDS-acrylamide gel. *Methods Enzymol.* **1972**, *26*, 3–27.
Weder, J. K. P. Chemistry of legume protease inhibitors and their use in taxonomy. *Qual. Plant Food Hum. Nutr.* **1985**, *35*, 183–194.
Whitaker, J. R.; Sgarbieri, V. C. Purification and composition of the trypsin-chymotrypsin inhibitors of *Phaseolus vulgaris* L. var Rosinha G2. *J. Food Biochem.* **1981**, *5*, 197–213.

Received for review June 27, 1988. Accepted November 1, 1988.

# Studies on Vegetables: Fiber Content and Chemical Composition of Ethanol-Insoluble and -Soluble Residues[1]

I. R. Siddiqui

The ethanol-insoluble residues from four vegetables were analyzed for moisture, nitrogen, starch, Klason lignin, ash, and uronic anhydride. The starch-free residues following enzymatic hydrolysis were recovered and analyzed for neutral sugars following acid hydrolyses and derivitization, as alditol acetates, by GLC. The sum of lignin, cellulose, and noncellulosic polysaccharides showed the dietary fiber contents of celery, parsnip, rutabaga, and squash to be 1.1, 4.7, 2.3, and 2.3%, respectively, on a fresh plant basis. The ethanol-soluble residues showed major amounts of glucose, myoinositol, and sucrose. The residue from celery in addition showed appreciable amounts of malic acid (butanedioic acid) and exorbitant amounts of mannitol. Fructose was not detected in any of the four residues.

Dietary fiber embraces largely those materials consisting of lignin/polysaccharide complexes (cellulose, hemicellulose, pectin) and associated substances resistant to digestion by the enzymes of the human gestrointestinal tract. The fiber originates mainly from the plant cell walls and is largely composed of carbohydrates. By definition it also includes some commercially available food additive types such as alginates, carrageenans, pectins, xanthan gum, xyloglucans (amyloids), exudate gums, dextrans, levans, and 1,3-$\beta$-D-glucans. Some of the cell wall polysaccharides often occur in association with proteins, including cell wall proteins (extensin), cuticular waxes, fats, and polyphenols, and carry esterified groups containing methanol, acetic acid, and phenolic acids. Phytate, oxalate, and inorganic constituents are sometimes also present (Theander and Aman, 1979).

Traditional gravimetric methods of analysis are not adequate. A critical review of available methods (Southgate et al., 1978) points to some of the difficulties inherent in the methodologies. The drawbacks associated with these methods have been adequately reported (Theander and Aman, 1979; Bittner et al., 1982; Selvendran and Dupont, 1984). Additional information has appeared in a book by James and Theander (1981) and a review by Asp and Johansson (1984). In recent years methods based on analysis of ethanol-insoluble residues with or without removal of starch by enzymatic digestion have been reported by the preceding authors and others (Englyst et al., 1982; Selvendran and Dupont, 1980; Selvendran, 1984). For more recent information on the analytical aspects, reference can be made to the Report of The Expert Advisory Committee on Dietary Fiber, Health and Welfare, Canada (1985) and a study of Theander and Westerlund (1986).

This paper describes an alternative approach to fiber analysis using ethanol-insoluble residues from four Canadian vegetables. A report on the ethanol-soluble lower molecular weight carbohydrates is also presented.

Food Research Centre, Agriculture Canada, Ottawa, Ontario, K1A 0C6 Canada.

[1] Contribution No. 761, Food Research Centre.

## MATERIALS AND METHODS

The method described in the following pages meets most of the requirements suggested by Southgate et al. (1978) for a procedure of dietary fiber analysis. The following outline summarizes the major steps:

**Preparation and analysis of residues**: preparation of 80% ethanol-insoluble residues in sufficiently large amounts to achieve proper sampling and to avoid possible variation in the values of percent dry matter; analysis of residues for moisture, protein, starch, Klason lignin, ash, and uronic anhydride.

**Removal of starch**: removal of starch by amyloglucosidase and analysis of starch-free residues for any residual starch by enzymatic hydrolysis using $\alpha$-amylase and amyloglucosidase followed by determination of glucose by an automated glucose oxidase procedure.

**Analysis of starch-free residue**: hydrolysis under predetermined optimum conditions followed by addition of D-allose (internal standard) at the neutralization stage in both 1 M and 72% hydrolyses; determination of the relative percent composition (by weight) of neutral sugars (corrected for detector response factors) as alditol acetates.

**Calculation of dietary fiber**: calculation of total neutral polysaccharides in residues B (Table II) from 72% hydrolysis results, cellulose from the difference in the value of glucose between 72% and 1 M sulfuric acid hydrolyses, neutral noncellulosic polysaccharides by difference, and dietary fiber content by multiplying the sum of cellulose, noncellulose neutral polysaccharide, uronic anhydride, and Klason lignin with the weight of resides A (Table II) divided by 100.

**Plant Material.** Celery (*Apium graveolens*), parsnip (*Pastinica sativa*), rutabaga (*Brassica mapibrassica*), and squash (*Curcibita maxima*) were obtained fresh from a local distributor. Parsnips, rutabaga, and squash were peeled and diced prior to extraction while only the stalks of celery were used.

**Chromatographic Methods.** Descending paper chromatography was performed on Whatman No. 1 paper with ethyl acetate–pyridine–water (8:2:1). Paper electrophoresis was performed with Whatman No. 3MM paper with borate/calcium chloride buffer (pH 9.2) (Haug and Larsen, 1961) at 800 V for 4 h. Detection was effected with aniline hydrogen phthalate.

Ethanol-soluble residues were additionally analyzed by paper chromatography using butanol–pyridine–water (10:3:3) and with 0.2 M borate buffer (pH 10) and 0.2 M acetate buffer (pH 5) with

0021-8561/89/1437-0647$01.50/0 Published 1989 by the American Chemical Society