was related to storage time and temperature. Storage at temperatures greater than 43.3 °C greatly accelerated detinning of the canned product. Juices stored for 12 weeks at 48.9 °C (120 °F) contained the highest contents of tin; only a sample from midseason, however, exceeded the 250-ppm tolerance limit for tin in canned foods. The possibility of consumption of a juice subjected to these extreme storage conditions is unlikely because this temperature-abused product is unpalatable (aged, rotten flavor) and has an objectionable appearance (dark brown). Whenever possible, high temperatures during the warehousing and transporting of canned SSOJ should be avoided because of the disproportionally higher rates of detinning at these elevated temperatures.

LITERATURE CITED

Adams, J. B.; Blundstone, H. A. W. In "The Biochemistry of Fruits and their Products"; Hulme, A. C., Ed.; Academic Press: New York, 1971; Vol. 2, Chapter 15.
American Can Company "The Sanitary Can"; American Can Co.: Barrington, IL, 1973.
Bielig, H. J.; Treptow, H. *Chem., Mikrobiol., Technol. Lebensm.* **1973**, *2*, 129.
Birdsall, J. J.; Derse, P. H.; Teply, L. J. *J. Am. Diet. Assoc.* **1961**, *38*, 555.
Bombara, G.; Azzerri, N.; Baudo, G. *Corros. Sci.* **1970**, *10*, 847.
Codex Alimentarius Commission "Report of the Twelfth Session", FAO/WHO, Rome, 1978, p 45.
Cruess, W. V. "Commercial Fruit and Vegetable Products", 3rd ed.; McGraw Hill: New York, 1948; Chapter 14.
Curl, A. L. *Food Res.* **1949**, *14*, 9.
Frankenthal, R. P.; Carter, P. R.; Laubscher, A. N. *J. Agric. Food Chem.* **1959**, *7*, 441.
Kefford, J. F.; McKenzie, H. A.; Thompson, P. C. O. *J. Sci. Food Agric.* **1959**, *10*, 51.
Koehler, E. L. *Corrosion (Houston)* **1961**, *17*, 93t.
Koehler, E. L.; Canonico, C. M. *Corrosion (Houston)* **1957**, *13*, 227.
Kohman, E. F.; Sanborn, N. H. *Ind. Eng. Chem.* **1928**, *20*, 76.
Lambeth, V. N.; Fields, M. L.; Brown, J. R. *Food Technol. (Chicago)* **1967**, *21*, 1516.
Lueck, R. H.; Blair, H. T. *Trans. Am. Electrochem. Soc.* **1928**, *54*, 257.
Mahadeviah, M.; Eipeson, W. E.; Balakrishran, K.; Sastry, L. V. L. *J. Food Sci. Technol.* **1975**, *12*, 234.
McHard, J.; Foulk, S. J.; Jorgensen, J. L.; Bayer, S.; Winefordnez, J. D. *ACS Symp. Ser.* **1980**, *No. 143*, Chapter 16.
McHard, J.; Foulk, S. J.; Winefordner, J. D. *J. Agric. Food Chem.* **1979**, *27*, 1326.
McHard, J.; Winefordner, J. D.; Ting, S. V. *J. Agric. Food Chem.* **1976**, *24*, 950.
Moore, E. L.; Atkins, C. D.; Huggart, R. L.; MacDowell, L. G. *Citrus Ind.* **1951**, *32*, 8, 11, 14.
Nagy, S. In "Citris Science and Technology"; Nagy, S.; Shaw, P. E.; Veldhuis, M. K., Eds.; Avi Publishing Co.: Westport, CT, 1977; Vol. 1, Chapter 13.
Omori, Y.; Takanaka, A.; Ikeda, Y.; Furuya, T. *Nippon Yakurigaku Zasshi* **1955**, *61*, 77.
Omori, Y.; Takanaka, A.; Tanaka, S.; Iketa, Y.; Furuya, T. *Shokuhin Eiseigaku Zasshi* **1973**, *14*, 69.
Riester, D. W.; Braun, O. G.; Pearce, W. E. *Food Ind.* **1945**, *17*, 742.
Rouseff, R.; Ting, S. V., submitted for publication in *J. Food Sci.*
Royo, Iranzo, J.; Grima, R. *Rev. Agroquim. Tecnol. Aliment.* **1973**, *13*, 326.
Saguy, I.; Mannheim, C. H.; Passy, N. *J. Food Technol.* **1973**, *8*, 147.
Ting, S. V. *Proc. Fla. State Hortic. Soc.* **1977**, *90*, 178.
Vaurio, V. W. *Corrosion (Houston)* **1950**, *6*, 260.
Veldhuis, M. K. In "Fruit and Vegetable Juice Processing Technology", 2nd ed.; Tressler, D. K.; Joslyn, M. A., Eds.; Avi Publishing Co.: Westport, CT, 1971; Chapter 2.
Willey, A. R. *Br. Corros. J.* **1972**, *7*, 29.

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Extraction and Isolation of Soluble and Insoluble Fiber Fractions from the Pinto Bean (*Phaseolus vulgaris*)

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A method was tested to separate the fiber portion of the pinto bean into 13 fractions. The method overcame many problems which make extraction of fiber from high-starch and/or high-protein products difficult. The lipid, fatty acid, and protein content associated with each fraction was investigated in both raw and cooked beans. Cooked beans contained more than twice the amount of soluble fiber than the raw pinto beans, while the cooking process reduced by one-third the extractable hemicellulose A and completely depleted the hemicellulose B. Extraction by this technique revealed a 50% decrease in the levels of lignocellulose and crude cellulose after the cooking process. Some protein contamination was found in all fiber fractions except the bound pectin. Lipids, as phospholipids, monoglycerides, diglycerides, triglycerides, and free fatty acids were found in all fiber fractions tested except at the lignocellulose level of the cooked beans.

Although there are techniques available for the detailed analysis of the structural macromolecular components of the plant cell wall, they are very time consuming, require elaborate, expensive equipment, and often are destructive of the molecules they seek to elucidate (Talmadge et al., 1973). To date, it is generally agreed that no single procedure is entirely satisfactory if one considers the complexity of dietary fiber in a normal mixed diet (Southgate, 1976).

Furda (1977) proposed a fiber extraction technique which for the most part was based on methodology long accepted by the scientific community. It included extraction techniques to isolate water-soluble fiber fractions.

During this study, several major changes were incorporated into Furda's original outline. Typical of the changes was the use of both amylolytic and proteolytic enzymatic

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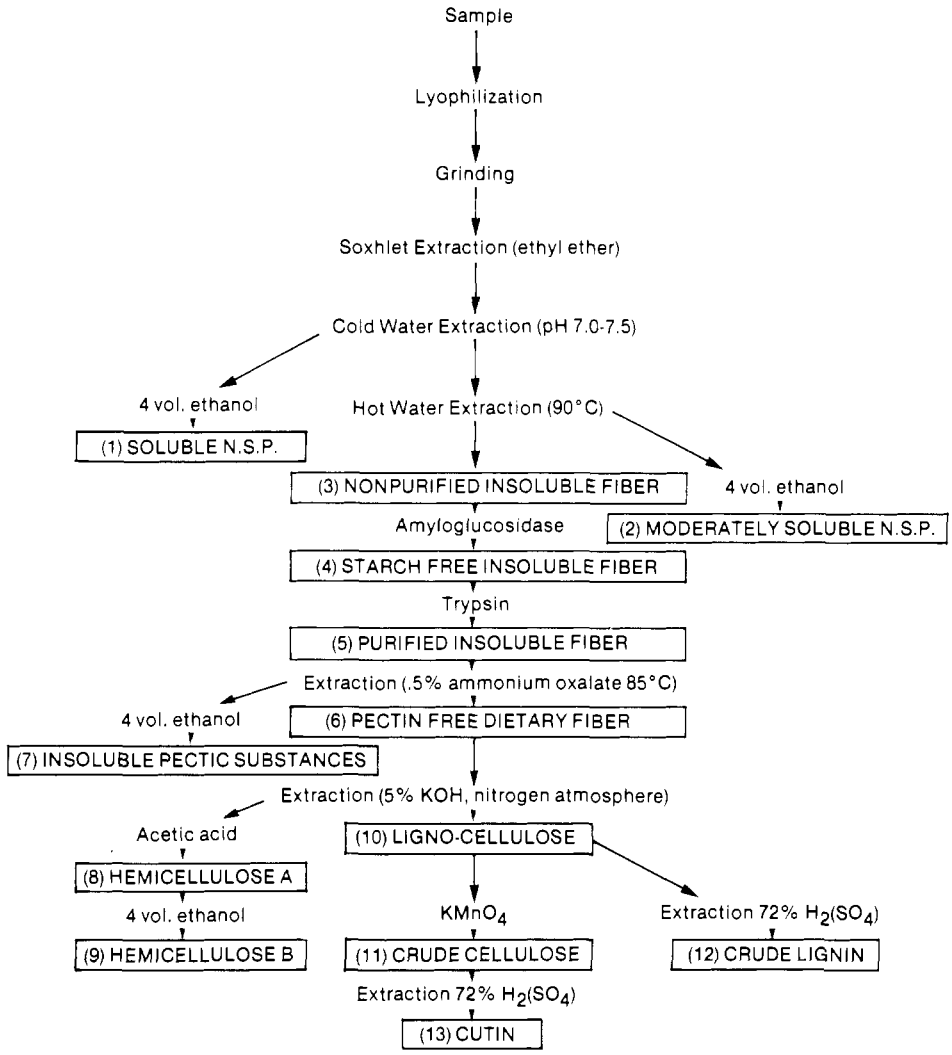

Figure 1. Fiber extraction and fractionation flow diagram.

digestive techniques in the treatment of the supernatants containing the highly and moderately soluble polysaccharides, in an effort to reduce contamination of these fractions by digestible polymers. The enzymatic removal of starch and protein from the insoluble fiber followed the methods developed by Elchazly and Thomas (1976) for high-starch, high-protein foods. Recovery of the insoluble pectic substances was accomplished with an ammonium oxalate extraction developed by Southgate (1976). The methods of Goering and Van Soest (1962) as modified by Elchazly and Thomas (1976) were utilized for the isolation of lignin, cellulose, and cutin.

Thus, the objective of this study was to test and further develop the fiber extraction procedure proposed by Furda (1977). Pinto beans were chosen as the test food because of their high starch (60%) and protein (20–25%) content (Watt and Merrill, 1963). Such products are said to be very difficult to extract by many of the fiber analysis techniques commonly used for high-fiber substances (Southgate, 1976).

PROCEDURE

Pretreatment of Beans. The fiber extraction method described below (Figure 1) incorporates the suggestions of Furda (1977) and all modifications made during the course of this study.

The beans, certified no. 114 pinto beans (proximate analysis of raw beans in Table I) grown under dry land conditions in Colorado, were handpicked of rocks and debris and then vacuum cleaned. They were divided into two 10-lb groups. One group was dried under vacuum at 20 °C until their weight was stabilized. The others were soaked in 4 times their weight for 10 h in cold water and then cooked according to custom at a slow simmer for 2 h. The cooking water was poured off and discarded, and the beans were rinsed with 30 L of 20 °C distilled water. These beans were then freeze-dried and packed in a dry nitrogen gas atomosphere. Both groups of beans were treated identically throughout the course of the experiment.

Table I. Proximate Analysis of Raw Pinto Beans

	% of raw bean
water	8.3
lipid	2.2
protein	22.9
carbohydrate	63.7
crude fiber	4.3
ash	3.9

Milling of Beans. The beans were first passed through a commercial coffee grinder, factory set for drip grind. They were then processed with a Wiley mill (Model No. 4) 3 times, until all passed through a mesh size of 0.5 mm. Heat during this process can cause an alteration of polymer structure (Southgate, 1976); therefore, this slow stepwise procedure was much favored to many quicker alternatives. Between and after grinding, all beans and bean fiber fractions were stored in dry nitrogen gas to prevent ab-

sorption of water from the environment and oxidation of lipids.

Extraction of Lipids. A 24-h Soxhlet extraction was performed twice by using reagent-grade diethyl ether. The beans were reground between extractions. The lipid extract was weighed and discarded. The residue was dried under vacuum to assure complete removal of solvent.

Isolation of Highly Soluble Complex Carbohydrates. The extracted residue from above was washed with water (pH 7.0–7.5) at room temperature and shaken vigorously for 6 h. The residue was saved. This extract contained several complex carbohydrate fractions that were later recovered along with some sugars, minerals, and water-soluble proteins.

Depolymerization and Removal of Starch and Protein. The supernatants from above were combined, and 2 mL of 0.2 M phosphate bufer (pH 6.9) was added along with 2 drops of saturated calcium carbonate solution followed by 3 mL of a 5% (w/v) suspension of amyloglucosidase (Sigma A 7255), 3 mL of a 2.5% solution of trypsin, and a few drops of toluene. The mixture was incubated at 55 °C for 18 h and then filtered.

Removal of Water-Soluble Proteins. Acidification of the above extract with hydrochloric acid to pH 4.5 and then heating to 90 °C for 5 min precipitated much of the protein (Whistler, 1965). The precipitate was centrifuged and discarded.

Recovery of Soluble Nonstarchy Polysaccharides. To the above extract was added 4 volumes of ethanol. The precipitate was centrifuged and lyophilized for later use. Water had to be added to the precipitate to encourage freezing before lyophilization. This technique was used throughout whenever an ethanol precipitate was to be lyophilized. This fraction represents product 1 in Figure 1.

Isolation of Moderately Soluble Complex Carbohydrates. The insoluble residue from the first water wash above was extracted with hot water. Portions of 1–2 g were extracted in a boiling water bath with 20 mL of 90 °C water to which 0.01 g of EDTA (disodium ethylenediaminetetraacetate) had been added. The extractions were carried out in 50-mL centrifuge tubes for 30 min each. Three extractions were performed, the mixture was centrifuged at 4000 rpm in a heated (80 °C) centrifuge (a hair dryer was used to heat the centrifuge before use), and the supernatant was pooled after each extraction. The residue called *nonpurified insoluble fiber* (product 3 in Figure 1) was lyophilized and saved.

The supernatant contained complex carbohydrate fractions that were recovered, starch which was depolymerized and discarded, and protein, most of which was precipitated and removed.

Depolymerization and Removal of Starch and Protein. This was accomplished as described under Isolation of Highly Soluble Complex Carbohydrates.

Removal of Water-Soluble Proteins. This was performed as outlined under Isolation of Highly Soluble Complex Carbohydrates.

Recovery of Moderately Soluble Nonstarchy Polysaccharides. This was performed as outlined in the previous section. This precipitate was centrifuged and lyophilized for later use and represents product 2 in Figure 1.

Nonpurified Insoluble Fiber. The first residue from the hot water extraction above was free from lipids, low molecular weight substances, and polymers soluble in water. It contained predominantly structural polymers. An aliquot of this material was saved and called nonpurified insoluble fiber. This insoluble fiber did contain complex starch and some insoluble protein which were removed as follows.

Removal of Starch. One gram of nonpurified insoluble fiber was placed in a 50-mL glass centrifuge tube. Twenty-five milliliters of distilled water was added, and the tubes were autoclaved for 1 h at 130 °C. After the tubes were cooled to 40 °C, 2.5 mL of 0.2 M phosphate buffer solution (pH 6.9), 5 mL of a 10% solution of amyloglucosidase (filtered before use), and a few drops of toluene were added. This was incubated with moderate shaking for 18 h at 55 °C and centrifuged for 10 min at 3000 rpm. The filtrate was discarded and the precipitate washed with water. An aliquot of this fraction, starch-free insoluble fiber (product 4 in Figure 1), was lyophilized and saved for later use.

Removal of Protein. To the starch-free insoluble fiber was added 25 mL of 2.5% trypsin (U.S. Biochemical) solution (filtered before use) and 5 mL of 1.0 M Sorensen's phosphate buffer (pH 8.0), plus a few drops of toluene. The mixture was incubated for 18 at 37 °C with frequent agitations. The tubes were centrifuged, and the residue was washed with 30 mL of distilled water. Then 20 mL of cold 3 N hydrochloric acid was added for 5 min to extract some of the organic acids and minerals. This was centrifuged, and the residue was washed with distilled water several times until acid was no longer detectable. The same process was repeated with ethyl alcohol, acetone, and diethyl ether. The residue was then dried at 45 °C. This protein- and starch-free insoluble fiber was considered to be a sample of purified insoluble fiber (product 5 in Figure 1).

Recovery of Insoluble Pectic Substances. A 3-g aliquot of purified insoluble fiber was extracted with 10 mL of a 0.5% (w/v) ammonium oxalate solution at 85 °C for 2 h. The extraction was repeated 4 times. The filtrates were combined and brought to a pH of 6.5 with hydrochloric acid. A precipitate was produced by adding 4 volumes of ethanol. The precipitate was then centrifuged and washed with 70% (w/v) ethanol (slightly acidified with HCl) and then with ethanol followed by acetone. The acetone was allowed to evaporate before the sample of insoluble pectic substances (product 7 in Figure 1) was lyophilized and saved. An aliquot of the residue after oxalate treatment represents pectin-free dietary fiber (product 6 in Figure 1) and was lyophilized and retained.

Recovery of Hemicelluloses A and B. The pectin-free dietary fiber was placed in a 50-mL glass-stoppered centrifuge tube to which enough 5% (w/v) potassium hydroxide solution was added to cover the sample (usually 10 mL). The flask was then flushed with nitrogen and shaken for 24 h. The residue was centrifuged at 3000 rpm for 10 min. The extraction was repeated 3 times. The residue lignocellulose (product 10 in Figure 1) was washed, dried, and saved for below. The filtrates were combined, and acetic acid was added dropwise until the solution was made "faintly" acid. The precipitate that formed was hemicellulose A (product 8 in Figure 1). The remaining filtrate was diluted with 4 volumes of ethanol to produce a second precipitate, hemicellulose B (product 9 in Figure 1).

Crude Cellulose. One-half the lignocellulose from above was extracted with a solution of potassium permanganate (Goering and Van Soest, 1970); all filtrates were discarded. Previously weighed crucibles were placed in a shallow enamel pan containing cold water to a depth of about 1 cm. The fiber in the crucibles was kept dry. About 25 mL of combined saturated potassium permanganate

and lignin buffer solution (2:1 by volume) was added to the crucibles. The level of water in the pan was adjusted to 2–3 cm to reduce the flow of solution out of the crucibles. A short glass rod was placed in each crucible to stir the contents, break lumps, and draw the permanganate solution up on the sides of the crucibles to wet all the particles. The crucibles were allowed to stand at 20–25 °C for 90 ± 10 min. More mixed permanganate solution was added, if necessary, making sure the purple color was present at all times.

The crucibles were placed into the filtering apparatus and sucked dry. They were placed in a clean enamel pan and filled no more than half full with demineralizing solution (Goering and Van Soest, 1970). The demineralizing solution was added directly to the crucibles in case the filtering was difficult. After about 5 min, the crucibles were sucked dry on the filter and refilled half full with demineralizing solution. The process was repeated if the solution was very brown. The sides of the crucibles were rinsed with solution from a wash bottle with a fine stream. The treatment was continued until the fiber was white.

The crucible was filled with and the contents were thoroughly washed with 80% ethanol and sucked dry, and the process was repeated twice more. The procedure was duplicated with acetone and then sucked dry.

The resultant crude cellulose (product 11 in Figure 1) was dried overnight and weighed.

Crude Lignin. The remaining half of the lignocellulose was placed in a Pyrex breaker to which was added 10 mL of a cold 72% solution of sulfuric acid. This was placed on a magnetic stirrer in a refrigerator at 0–4 °C for at least 30 h. To this was added "quickly" 30 mL of cold distilled water. The residue crude lignin (product 12 in Figure 1) was washed with distilled water until no acid was detectable. The washing was then continued with acetone and diethyl ether after which the lignin was dried at 100 °C.

Cutin. A portion of the unwashed crude cellulose was treated exactly as described above with a 72% solution of sulfuric acid. The residue was cutin (product 13 in Figure 1).

Lipid Determinations. Fractions were extracted with 2:1 chloroform–menthanol by the method of Folch. Extracts were evaporated to dryness with nitrogen and weighed. Lipids were resuspended in chloroform to a concentration of 2.5 mg of lipid per 50 μL. A 50-μL aliquot of this solution was applied to a precoated silica gel thin-layer chromatographic plate. All chromatographic plates were developed in a solution of hexane, diethyl ether, and formic acid (80:20:2) for 1 h. When the plates were sprayed with a 50% solution of sulfuric acid and heated to 100 °C, the various lipid fractions appeared. Plates were analyzed with a recording Beckman "Analytrol" densitometer to determine relative concentrations of the various lipid fractions. Plates that were not visualized with sulfuric acid were scraped after separation, and the scrapings were extracted with methanol and stored at −20 °C until ready for analysis by gas chromatography.

Gas Chromatography of Fatty Acids. C-15 fatty acid was added as an internal standard, and then saponification was performed by heating lipid extracts with methanolic KOH for 1 h at 70 °C. Fatty acids were extracted with petroleum ether after the samples were cooled at 4 °C and acidification to pH 1. Methylation of the fatty acids was accomplished by heating with boron trifluoride–methanol (Applied Science Laboratories). The resulting esters were extracted with petroleum ether and evaporated to the appropriate concentration for analysis with gas chromatography. The fatty acid analysis was performed on a Hewlett Packard 5830A instrument equipped with a flame ionization detector. A 15% diethylene glycol succinate on 80–100 mesh Chromosorb W (Applied Science Laboratories) packing in a 200 × 0.32 cm stainless steel column was used for separation. The carrier gas was helium at a flow rate of 20 mL/min. The injection port temperature was maintained at 275 °C and the detector temperature at 300 °C. The temperature of the column itself was programmed to climb from 170 to 210 °C at a rate of 4 °C/min.

Table II. Particle Size Distribution of Raw and Cooked Pinto Beans after Grinding

raw pinto beans, mesh size	% in each particle size category	
	raw	cooked
>40	22.4	1.4
>60	38.4	3.1
>80	33.1	45.5
>100	3.0	18.9
>200	0.0	15.4
>200	0.0	10.7

Protein Determinations. Two methods were used to determine protein. The Kjeldahl method was the method of choice for nitrogen with a factor of 6.25 to convert nitrogen percent to protein. A protein–dye binding method based on the color change of Coomassie Brilliant Blue G-250 when it binds to the basic groups of the protein molecule provided an excellent method for determination of low concentrations of soluble protein in polysaccharide solutions (Bradford, 1976).

RESULTS

Particle Size. The raw and cooked beans were ground as described earlier. A 150-g sample of each was then subjected to sieving to determine particle size distribution. As can be seen from Table II, cooking affected the structure of the pinto bean in such a way as to allow the grinding technique used in this method of extraction to produce a smaller average particle size. Over 95% of the particles from the cooked pinto bean passed through a 60-mesh screen, whereas in the case of the raw pinto bean the 60-mesh sieve allowed less than 40% of the particles to pass through.

The raw beans were much more difficult to grind than the cooked beans. A great deal of care was taken not to allow the temperature of the grinder to exceed 50 °C. After the first fat extraction, the raw beans could be reground much more easily; however, the average particle size of the raw beans was still greater than that of the cooked beans. Particle size has been discussed as a possible important parameter for fiber analysis (Southgate, 1976).

Lipid Content. It was expected that the two diethyl ether Soxhlet extractions would not allow significant lipid to remain in the sample. Particle size did not seem to have a great effect on the lipid extraction values of ether the cooked or raw beans. However, as shown in Table III, the Folch extraction did reveal that lipid remained in the sample after the water extractions. The increased lipid percent of the pectin-free dietary fiber was due to removal of protein and starch by enzymes that increased the concentration of the lipid which remained. The drastic reduction in lipid at the lignocellulose level may be attributed to the saponification effect of the 5% potassium hydroxide solution used to extract the hemicellulose. The separation of the various lipid extracts by thin-layer chromatography as shown in Table III revealed a very significant trend, which was that there was an increase in the relative pro-

Table III. Lipid Content of Pinto Bean Fiber Fractions

	total lipid (Soxhlet), %	total lipid (Folch), %	phospholipid as % of total lipid (TLC)	mono- and diglycerides as % of total lipid (TLC)	free fatty acids as % of total lipid (TLC)	triglycerides as % of total lipid (TLC)	sitosterol and sitosterol ester (TLC), %
cooked pinto beans	2.5	-					
nonpurified insol fiber		0.38	13.8	14.0	1.6	46.7	25.2
pectin-free dietary fiber	-	1.61	9.1	15.2	26.3	35.7	13.4
lignocellulose	-	0.0	-	-	-	-	-
raw pinto bean	2.9	-	12.0	4.4	2.1	59.4	20.8
insol fiber	-	0.51	35.3	6.4	7.0	24.9	18.5
pectin-free dietary fiber	-	1.52	12.7	9.3	34.7	28.6	8.1
lignocellulose	-	0.13	9.3	10.5	25.0	27.2	21.5

Table IV. Fatty Acid Content of Lipid Extracts of Pinto Bean Fiber Fractions

	composition of methyl esters, % (area % of GLC)				
source[a]	14:0	16:0	18:0	18:1	18:2
raw pinto bean oil					
area A	2.8	9.1	12.5	14.9	24.9
area B	2.8	15.1	4.0	12.3	48.0
area C					
area D	1.7	6.8	4.2	15.9	31.3
raw nonpurified insol fiber					
area A	2.2	10.3	7.0	20.4	40.0
area B	5.4	10.0	4.4	13.6	43.0
area C					
area D	3.9	4.7	8.0	16.9	58.0
raw pectin-free dietary fiber					
area A	4.1	16.3	7.6	13.0	33.6
area B	11.4	7.5	6.9	15.9	43.8
area C	0.9	5.7	19.1	23.5	30.0
area D	0.4	6.9	5.2	13.8	66.8
raw lignocellulose					
area A	0.2	18.1	9.3	15.6	37.0
area B	3.7	8.4	8.3	18.0	46.6
area C	0.8	9.7	21.4	25.6	10.2
area D	1.9	3.0	6.3	15.9	28.1

[a] Areas: (A) phospholipid; (B) mono- and diglycerides; (C) free fatty acids; (D) triglycerides.

Table V. Protein Analysis of Pinto Bean Fiber Fractions

	% protein (dye binding)	% protein (Kjeldahl; N × 6.25)
raw pinto bean		19.7
sol N.S.P	25.3	21.0
sol N.S.P. after trypsin	2.3	
moderately sol N.S.P.	15.5	11.8
moderately sol N.S.P. after trypsin	1.6	
nonpurified insol fiber	7.9	18.3
starch-free fiber	10.2	21.9
purified insol fiber	5.6	7.1
pectin-free fiber	6.3	6.9
insol pectin	0.0	
lignocellulose	6.9	4.6
cooked pinto bean		22.6
sol N.S.P.	23.9	
sol N.S.P. after trypsin	1.9	
moderately sol N.S.P.	16.9	
moderately sol N.S.P. after trypsin	1.3	
nonpurified insol fiber	7.8	18.7
starch-free fiber	10.4	20.8
purified insol fiber	5.5	4.8
pectin-free fiber	6.1	5.4
insol pectin	0.0	
lignocellulose	6.7	

portion of total lipid attributable to free fatty acid at the expense of the triglyceride fraction the digestion of protein by trypsin. It is reasonable to expect some free fatty acid to be bound to the protein fraction of the bean but why these fatty acids were not liberated during the enzyme treatment itself or the solvent wash that followed remains unknown. It is also important to note that the gas–liquid chromatographic analysis of the various lipid extracts (Table IV) showed that the majority of fatty acid present in the raw pinto bean was linoleate, yet the relative proportion of oleate to linoleate in the free fatty acid fraction (area C) of the enzyme-treated fiber fractions of the raw pinto bean was higher relative to the oleate/linoleate ratios of other lipid fractions regardless of treatment. Otherwise, the fatty acid distribution of the various fiber fractions was rather consistent from one lipid area to the next, independent of the fiber moiety in which it exists.

Protein Content. The dye binding method for protein analysis seemed to work quite well for the soluble fractions, but results with the insoluble fiber fractions were much lower than the protein results as determined from Kjeldahl nitrogen content by using a protein conversion factor of 6.25 for beans (Table V). It is expected that nonprotein nitrogen was falsely represented as protein by the Kjeldahl method. The large percentage of protein in the soluble nonstarchy polysaccharide fractions indicated a high degree of impurity. The introduction of a small amount of trypsin to the amyloglucosidase digestion reduced nitrogen to a more reasonable concentration. It was of interest that the water extractions did remove over 20% of the protein from both the cooked and raw beans. The dye binding technique and the Kjeldahl method show an increase in protein (from 7.9 to 10.2%) and nitrogen (from 2.9 to 3.5%), respectively (Table V), after the enzymatic digestion of the starch component of the insoluble fiber of both the raw and cooked beans. This is actually less than the increase that would be expected since the mass of both the raw and cooked insoluble fiber was reduced by over 50% by the amylolytic enzyme (Table VI), thus indicating the loss of some protein and possibly other nitrogen during this step. The persistence of protein beyond the trypsin treatment of the starch free fiber is consistent with recent reports in the literature showing protolytic resistance in a portion of the major storage protein of the pinto bean (Ryan and Romero, 1978). However, it would probably be unwise to consider the protein present after dietary fiber to be a valie in vitro counterpart to indigestible protein.

Results of Fiber Analysis of Pinto Beans. The results of pinto bean fiber analysis using the method described in this study were compared to corresponding results obtained by Elchazly and Thomas (1976) (Table VI). The means of the data collected from individual extractions were tested for significance. The equality of variances of the raw vs. cooked data means being tested was determined by using an F test for equality for variances. In cases where variances were unequal, a t test assuming unequal variances was used. Equal variances indicated the use of a t test, assuming equal variances.

Table VI. Results of Fiber Analysis of Raw and Cooked Pinto Beans (Expressed in % of Dry Product Weight)[a]

	raw pinto beans	Elchazly and Thomas (1976) (raw)	cooked pinto beans
sol N.S.P.	1.04 ± 0.13 (10)[a b]		2.12 ± 0.22 (10)[b c]
moderately sol N.S.P.	0.42 ± 0.04 (10)[a]		0.12 ± 0.02 (10)[b c]
nonpurified insol fiber	50.89 ± 1.43 (10)[a]		73.98 ± 1.57 (10)[b c]
starch-free fiber	24.70 ± 1.05 (9)[a]		24.93 ± 0.89 (10)[a]
purified insol fiber	18.89 ± 0.73 (6)[a]	14.8	17.37 ± 0.67 (6)[a]
pectin-free fiber	14.73 ± 0.24 (6)[a]		12.70 ± 0.66 (6)[a]
insol pectin	0.71 ± 0.11 (6)[a]		0.40 ± 0.05 (6)[b]
hemicellulose A	3.19 ± 0.39 (4)[a]		1.96 ± 0.35 (4)[b]
hemicellulose B	0.66 ± 0.07 (4)[a]	4.0	0.00 ± 0.00 (4)[b]
lignocellulose	10.03 ± 0.27 (4)[a]		5.78 ± 0.18 (4)[b c]
crude cellulose	6.61 ± 0.40 (4)[a]	9.0	3.19 ± 0.75 (4)[b]
crude lignin	1.53 ± 0.18 (4)[a]	1.8	1.09 ± 0.25 (4)[a]
cutin	1.17 ± 0.08 (4)[a]		0.91 ± 0.17 (4)[a]

[a] Mean ± SEM for (n) determinations. [b] Values within a row with the same roman superscript letter do not differ significantly ($P > 0.05$). [c] Significantly different from raw extraction at $P < 0.001$.

Statistical analysis showed that more than twice the amount of soluble N.S.P. fiber was extracted from the cooked pinto bean (2.12 ± 0.22%) as from the raw bean (1.04 ± 0.13%) ($P = 0.001$). When the beans were cooked, over 16% of their weight was lost when the cooking water was discarded. The concentrating effect of this step could account for an increase in the proportion of fractions that follow in succession. However, it would seem obvious that water-soluble fractions would be lost with the cooking water, even though the bean was intact and not ground at this point. Two hours of boiling may have caused modifications in certain constituents of other fiber fractions that made them more soluble when they were exposed to water after the grinding process. This would be supported by the disappearance of nearly 75% of the cooked moderately soluble N.S.P. (0.12 ± 0.02%) as compared to the raw moderately soluble N.S.P. (0.42 ± 0.04%) ($P = 0.001$) and an insignificant difference ($P = 0.05$) between the starch-free purified insoluble fiber and pectin-free fiber fractions even though they would be expected to be higher in the cooked beans on a dry weight basis because of the concentrating effect on the cooking process. Only half as much insoluble pectin was extracted from the cooked bean (0.40 ± 0.50%) as from the raw bean (0.71 ± 0.11%) ($P = 0.05$). The cooking process may be responsible for this since cooking considerably weakens the cell walls and insoluble pectin is thought to be associated with the cell wall (Aspinall, 1970). The concentrating effect of cooking should have resulted in a marked increase in the hemicellulose A and B fractions of the cooked beans over the raw beans. The results were the opposite of those expected, with hemicellulose being reduced by one-third in the cooked hemicellulose A fraction (1.96 ± 0.35%) as compared to the corresponding raw fraction (3.19 ± 0.39%) ($P = 0.05$) and hemicellulose B completely disappearing from the cooked bean. It is postulated that several factors may be working together to produce this effect. The most important factor may be the much smaller size of the average cooked bean particle (Table II). It is not uncommon to lose some hemicellulose when enzyme methods are used, particularly those using amylases (Southgate, 1976). The greater the contact of hemicellulose with amylase the greater the chance of 1–4 linkages being broken with possible release of soluble oligosaccharide segments of the hemicellulose molecules. The same rationale may hold true for cellulose, thus accounting for the 50% decrease in the levels of *lignocellulose* and crude cellulose in the cooked beans. The lignin and cutin fractions of cooked beans were not significantly different from those of raw beans ($P = 0.05$). Lignin and cutin are both quite resistant to the effects of enzymes, and it was not expected that their concentrations would change during the cooking process.

LITERATURE CITED

Aspinall, G. O., in "Polysaccharides", Pergamon Press, New York, 1970, p 14.
Bradford, M. M., *Anal. Biochem.* **72**, 248 (1976).
Elchazly, M., Thomas B., *Z. Lebensm.-Unters.-Forsch.* **162**, 329 (1976).
Furda, I., *Cereal Foods World* **22**, 252 (1977).
Goering, H. K., Van Soest, P. J., *Am. Assoc. Cereal Chem. Meth.*, 32-15 (1962).
Goering, H. K., Van Soest, P. J., USDA, Agriculture Handbook No. 379, 1970.
Ryan, D. S., Romero, J., *J. Agric. Food Chem.* **26**, 784 (1978).
Southgate, D. A. T., in "Determination of Food Carbohydrates", Applied Science, London, 1976, pp 48–85.
Talmadge, K. W., Keegstra, K., Bauer, W. D., Aldersheim, P., *Plant Physiol.* **51**, 158 (1973).
Watt, B. K., Merrill, A. L., USDA, Composition of Foods, Agricultural Handbook No. 8, 1963.
Whistler, R. L., in "Methods in Carbohydrate Chemistry", Academic Press, New York, 1965, pp 3–59.

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