Received for review August 2, 1982. Revised manuscript received February 7, 1983. Accepted February 18, 1983. This investigation was supported by the New Crop Development Fund, Food Production and Marketing Branch, Agriculture Canada, the National Science and Engineering Council of Canada, a Manitoba Department of Agriculture grant to the Faculty of Agriculture, and the University of Manitoba. A CIDA graduate student assistantship was held by D.S.M.

Characteristics of Pindak Bean Starch

Mark L. Dreher,* G. Padmanaban, and Richard A. Frazier

Pindak bean (a cross between the pinto and Japanese bush bean) starch was characterized and compared to pinto bean and HRW wheat starches. Pindak bean flour yielded 43.5% starch compared to 40.0% for pinto bean flour. The pindak and pinto bean starches were irregular and somewhat elliptical with a few small, round granules. The amylose content of the pindak bean was 27.2% for the colorimetric method and 33.6% for the potentiometric method. The solubilities at 85 °C for all starches were similar, and the swelling power at 85 °C was more variable, with pindak starch having the highest value. The pindak and pinto bean starches had a type C viscosity pattern with viscosities higher than that of wheat starch. The initial pasting temperature for pindak starch was 77.5 °C. Further studies need to be done on the food uses of dry edible bean starches.

The pinto bean is one of the major dry edible beans grown in the United States. The pindak bean, a cross between the pinto bean and the Japanese bush bean, has been recently released by North Dakota State University (Schneiter et al., 1981). Pindak beans appear to have a more uniform size distribution and a greater degree of disease resistance than pinto beans. Consequently, pindak beans have potential for large-scale commercial production.

Starch has been shown to be a major component of legumes (Schoch and Maywald, 1968). Starch is used extensively throughout the food industry as a filler, extender, thickener, stabilizer, and texture modifier (Wurzburg, 1968). A nontraditional method for utilizing beans may involve its fractionation into protein and starch component by air classification (Satterlee, 1981). This process could be especially important for developing food uses for culled beans. According to Satterlee (1981), approximately 100 million pounds of low-cost culled beans are available each year in the United States. The objective of this study is to evaluate the characteristics of pindak bean starch and compare them with those of pinto and wheat starch.

EXPERIMENTAL PROCEDURES

The pindak beans were obtained from North Dakota State University (1981 crop). The pinto beans were obtained commercially (1981 crop). Bean starches were isolated by using method A of Schoch and Maywald (1968). The starch was air-dried (3 days) and passed through a 70-mesh sieve. The standard starch source was commercially isolated hard red winter (HRW) wheat.

Standard AOAC (1980) methods were used to determine starch moisture, nitrogen, and ash. Acid detergent fiber (ADF) was determined by the method of Goering and Van Soest (1970). Bean starch content was determined by a modification of the Osborne and Voogt (1978) method using amyloglucosidase. Starch digestibility of pindak starch with α-amylase (Type VII-A, Sigma Chemical Co.) is determined by a modification of methods from Rao (1969) and Kayisu and Hood (1979). The digestion products glucose and maltose were determined by using HPLC analysis with a carbohydrate analysis column (Waters Associates), mobile phase 70:30 (acetonitrile–water), flow rate 1.8 mL/min, and detection using a 401 refractometer. The amylose content (blue value) was determined by the method of Williams et al. (1970). Iodine affinity for the pindak starch was determined by potentiometric titration (Schoch, 1964). Swelling power and solubility of the starches were determined at 85 °C according to the method of Schoch (1964). Water binding capacity of the starches was determined by the procedure of Medcalf and Gilles (1965). Pasting curves at 12% starch in distilled water were determined with a Brabender VISCO/amylo/GRAPH by using a standard cycle of heating from 25 to 95 °C, holding at 95 °C for 15 min and cooling to 50 °C (Naivikul and D'Appolonia, 1979). The size and shape of each starch were studied with a SO Spencer light microscope equipped with a 35-mm Kodak camera. Photomicrographs were taken at a magnification of 100 by using normal light for the measurement of granule size.

RESULTS AND DISCUSSION

The starch yield and chemical data on the starch are shown in Table I. The yield of starch (dry weight basis) from pindak bean flour was 43.5%, which was similar to the starch yield from pinto bean flour (40.0%). The starch yields in this study were comparable with the legume values obtained by Naivikul and D'Appolonia (1979). The dry bean starch levels were lower than the 70–80% levels obtained for wheat flour (Pomeranz, 1971). The nitrogen content of the starches was low (0.01–0.07%) with values similar to those reported by Naivikul and D'Appolonia (1979). The oil content was extremely low and not reported due to the high degree of error. The ash content was low (0.01–0.02%) for the laboratory-isolated bean starches with the commercially available starch having a higher ash content (0.61%). The higher ash content of the wheat starch is probably due to the variation in industrial vs. laboratory isolation methods. The acid detergent fiber content of the starches ranged from 0.03 to 0.08%, which was slightly lower that the values reported by Naivikul and D'Appolonia (1979). Part of the difference in compositional data from previous work may be due to the methods of analysis used.

North Dakota State University, Agricultural Experiment Station, and College of Home Economics, Food and Nutrition Department, Fargo, North Dakota 58105.

0021-8561/83/1431-0844$01.50/0

Table I. Compositional Data on Starches

starch source	starch yield, %	nitrogen, %	ash, %	acid detergent fiber, %
pindak	43.5 ± 1.5	0.01 ± 0.00	0.01 ± 0.00	0.03 ± 0.01
pinto	40.0 ± 2.0	0.07 ± 0.00	0.02 ± 0.00	0.08 ± 0.02
HRW wheat		0.04 ± 0.00	0.61 ± 0.02	0.04 ± 0.02

Table II. Granule Size of Starches

starch source	width, μm	length, μm
pindak	12-25	14-40
pinto	12-25	14-35
HRW wheat	10-20	12-40

Table III. Physicochemical Properties of Starches

starch source	amylose, %	water binding capacity, %	solubility,[a] %	swelling power,[a] %
pindak	27.2 ± 1.92	88.7 ± 3.4	6.3 ± 0.5	9.7 ± 1.0
pinto	24.2 ± 2.41	98.5 ± 1.9	6.5 ± 0.8	6.8 ± 0.3
HRW wheat	24.1 ± 0.60	79.2 ± 0.8	6.3 ± 1.1	8.3 ± 0.3

[a] At 85 °C.

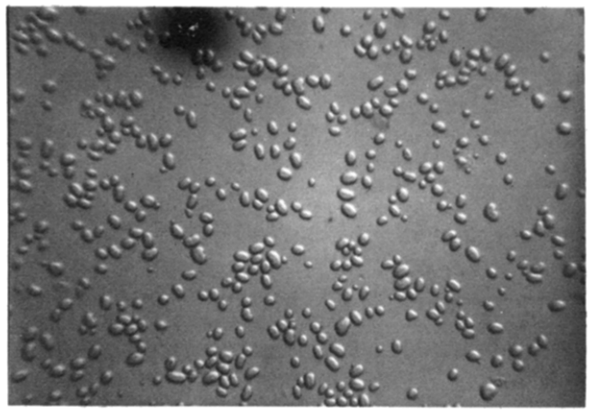

Figure 1. Starch granules isolated from Pindak beans (60×).

The range in granule size of the isolated legume starches is shown in Table II, with the shape of the pindak starch shown in Figure 1. The size and shape of starch granules are usually characteristic of the species of the plant and its maturity (Manners, 1974). The pindak and pinto bean starches were irregular and somewhat elliptical with a few small, round granules. The results of the granular size and shape of the pinto bean starch was comparable to those of a previous study (Naivikul and D'Appolonia, 1979). The wheat starch granules were a mixture of large to intermediate spherical granules which were about the same size as the bean starch granules.

The physicochemical properties of the starches are shown in Table III. The amylose content of the bean starches using the colorimetric method ranged from 24.2 to 27.2%, which was comparable with previous results (Naivikul and D'Appolonia, 1979) and similar to the range reported by Medcalf and Gilles (1965) for wheat starch (23.4–27.5%). The iodine affinity value for pindak starch was 6.3, giving a calculated amylose content of 33.6%, similar to amylose values for black bean starch reported by Lai and Varriano-Marston (1979). The discrepancy in pindak amylase results can be explained by differences in the methods used. The water binding capacity values of the starches were highly variable. The pindak starch had similar values than those previously reported by Naivikul and D'Appolonia (1979), but the pinto bean starch values were greater than those values previously reported. The water binding capacity for the wheat starch was slightly lower than the previous values reported by Medcalf and Gilles (1965). The solubility and swelling of the starches were evaluated at 85 °C. The solubilities of pindak, pinto, and wheat starches were approximately the same (6.3–6.5%). The bean starches had lower solubility than values previously reported for legume starches (Schoch and Maywald, 1968). Pindak bean starch had the highest swelling power, wheat starch intermediate swelling power, and pinto starch the lowest. The pindak bean starch swelling power was approximately equal to the swelling power of navy and garbanzo beans and lentils reported by Schoch and Maywald (1968).

Table IV. Pasting Properties of Starches

starch source	initial pasting temp, °C	peak height, BU	95 °C height, BU	15-min height, BU	50 °C height, BU
pindak bean	77.5		680	780	1200
pinto bean	74.5		550	710	1120
HRW wheat	80.5	600	410	600	1000

The pasting properties of the bean and wheat starches are shown in Table IV. The terminology used to express amylogram results has been previously described (Medcalf and Gilles, 1966). The pindak and pinto bean starches had a type C viscosity pattern with no pasting peak but a continual rise in viscosity throughout the heating period. The starch isolated from pindak and pinto bean flour had similar pasting properties with pindak bean starch having a slightly higher initial pasting temperature. The bean starches showed higher viscosity than the wheat starch viscosity values which agreed with Lineback and Ke (1975). Since different initial pasting temperatures occur for different concentrations of starches, direct comparison between the amylogram data in this study and previously reported data is complex. However, the amylogram curves for the starches in this study were similar to those reported by Naivikul and D'Appolonia (1979). No values are reported for peak viscosity with bean starch, because no distinct peak was obtained as is usually the case with wheat starch. The bean starches behaved similarly to cross-bonded starches with stabilized, swollen granules against mechanical fragmentation, giving constant or increasing viscosity during cooking. Further studies are warranted for the possible incorporation of pindak and pinto bean starch into infant foods, puddings, and pie fillings.

Pindak starch showed a slow rate of in vitro starch hydrolysis (16.2%). This rate of hydrolysis compared favorably with values reported for legumes by Reddy et al. (1982). The apparent digestibility of pindak bean starch in laboratory animals needs further investigation.

Registry No. Starch, 9005-25-8; amylose, 9005-82-7.

LITERATURE CITED

AOAC "Methods of Analysis"; Association of Official Analytical Chemists: Washington, DC, 1980.
Goering, H. K.; Van Soest, P. J. *U.S., Dep. Agric., Agric. Handb.* **1970**, *No. 379.*
Kayisu, K.; Hood, L. F. *J. Food Sci.* **1979**, *44* (6), 1728.
Lai, C. C.; Varriano-Marston, E. J. *J. Food Sci.* **1979**, *44* (2), 528.
Lineback, D. R.; Ke, C. H. *Cereal Chem.* **1975**, *52*, 334.
Manners, D. J. "Essays in Biochemistry"; Academic Press: New York, 1974; Vol. 10, p 37.
Medcalf, D. G.; Gilles, K. A. *Cereal Chem.* **1965**, *42*, 558.

Medcalf, D. G.; Gilles, K. A. *Staerke* **1966**, *4*, 101.
Naivikul, O.; D'Appolonia, B. L. *Cereal Chem.* **1979**, *56* (1), 24.
Osborne, D. R.; Voogt, P. "The Analysis of Nutrients in Foods"; Academic Press: New York, 1978.
Pomeranz, Y., Ed. "Wheat Chemistry and Technology"; American Association of Cereal Chemists: St. Paul, MN, 1971.
Rao, S. P. *Indian J. Med. Res.* **1969**, *57* (11), 2151.
Reddy, N. R.; Salunkhe, D. K.; Sathe, S. K. *CRC Crit. Rev. Food Sci. Nutr.* **1982**, *Jan*, 48.
Satterlee, L. D. *Food Technol. (Chicago)* **1981**, *35* (6), 58.
Schneiter, A. A.; Burke, D. W.; Venette, J. R. *N.D. Farm Res.* **1981**, *38* (5), 6.
Schoch, T. J. "Methods in Carbohydrate Chemistry"; Academic Press: New York, 1964; pp 106, 157.
Schoch, T. J.; Maywald, E. C. *Cereal Chem.* **1968**, *45*, 564.
Williams, P. C.; Kuzina, F. D.; Hlynka, I. *Cereal Chem.* **1970**, *47*, 411.
Wurzburg, O. B. "Handbook of Food Additives"; CRC Press: Cleveland, OH, 1968; p 377.

Received for review September 7, 1982. Revised manuscript received March 21, 1983. Accepted March 30, 1983. Published with the approval of the Director of the North Dakota Agricultural Experiment Station as Journal Article No. 1216.

Comparative Study of Soybean Plasteins Synthesized with Soluble and Immobilized α-Chymotrypsin

Cosimo Pallavicini,* Angelo Dal Belin Peruffo, and John W. Finley

Plasteins were prepared from low molecular weight peptides of a peptic digest of soybean protein. The plasteins were fingerprinted on silica gel by a combination of electrophoresis and TLC, eluted, hydrolyzed, and subjected to amino acid analysis. The results indicated that the plasteins from soluble α-chymotrypsin were richer in glycine, valine, leucine, and serine than the plastein prepared from immobilized α-chymotrypsin. Plasteins prepared from immobilized α-chymotrypsin were rich in glutamic acid, lysine, alanine, and threonine, suggesting a much more hydrophilic plastein than prepared from the soluble form of the enzyme.

In a previous study, Pallavicini et al. (1980) reported that plasteins prepared from several sources exhibited slightly different patterns when subjected to isoelectric focusing. The most significant differences were between plasteins prepared from soluble and immobilized α-chymotrypsin. The differences occurred in plasteins prepared form hydrolysates of soybean, alfalfa, and wild grass leaf protein. The differences in mobility were interpreted to mean that there were slight differences in the isoelectric points of plasteins prepared from soluble and immobilized α-chymotrypsin. Plasteins offer considerable potential for control of functional and nutritional characteristics and for this reason they are of great interest to the food industry. Several reports emphasize the interest and potential of plasteins (Pallavicini et al., 1980; Yamashita et al., 1970a,b; Onoue and Riddle, 1973; Savangikar and Joshi, 1979; Hofsten and Lalasidis, 1976; Eriksen and Fagerson, 1976).

In this study, plasteins prepared from peptic digests of soy protein are compared when the plastein are made by using either soluble or immobilized α-chymotrypsin. Comparisons are made by fingerprinting the plasteins on TLC and by amino acid analysis of the new peptides recovered from the fingerprinting.

METHODS AND MATERIALS

Protein Extraction. Soybean flour (12.5% moisture) was purchased from a local supplier and was stored at −20 °C until used. The extraction of the proteins from soy flour and the hydrolysis with pepsin (pH 1.6–1.8, 40 °C for 40 h) was done as previously described by Pallavicini et al. (1980). The low molecular weight peptide fraction of the hydrolysate was prepared by dialysis of the peptic digest against water. Spectrapor membrane tubing with a molecular exclusion of 3500 was used for the separation. The diffusate was concentrated in vacuo at 40 °C. The concentrate was then freeze-dried. The freeze-dried material was used at a 30% (w/v) concentration as the substrate for plastein synthesis with either soluble or immobilized α-chymotrypsin.

Plastein Preparation with Soluble α-Chymotrypsin. The 30% low molecular weight peptide fraction was filtered through Whatman No. 4 paper and the solution was incubated with α-chymotrypsin (salt free, type II, from Sigma Chemical Co., St. Louis, MO). The following conditions were used: substrate concentration 30% (w/v); enzyme/substrate ratio 1/100; pH 5.0; incubation temperature 38 °C for 6 h. The water-insoluble plastein products were purified by dialysis for 3 days at 5 °C according to Noguchi et al. (1975). The dialysis was carried out in a Spectrapor membrane tubing with an exclusion limit of 8000. Dialysis was carried out against four changes of distilled water. At the end of dialysis the contents of the bag were freeze-dried and used for the fingerprinting.

Plastein Preparation with α-Chymotrypsin Immobilized on Chitin. The enzyme immobilization procedure was as previously described by Pallavicini et al. (1980). Briefly the α-chymotrypsin was immobilized on 20–30-mesh chitin with glutaraldehyde and packed in a jacketed column. Column operation conditions were as previously described. The reaction product containing fractions from the column were pooled and dialyzed to retain only the water-insoluble fractions. Dialysis conditions were the same as for the plasteins prepared by soluble α-chymotrypsin. At the end of dialysis the contents of the bag were freeze-dried and fingerprinted.

Istituto di Industrie Agrarie, Via Amendola 165, 70126 Bari, Italy (C.P.), Istituto di Chimica Agraria e Industrie Agrarie, Via Gradenigo 6, 35100 Padova, Italy (A.D.B.P.), and Department of Pediatrics, University of Iowa Hospitals, University of Iowa, Iowa City, Iowa 52242 (J.W.F.).

0021-8561/83/1431-0846$01.50/0