Production and Functionality of Starches and Protein Isolates from Legume Seeds (Field Peas and Horsebeans)

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ABSTRACT

Technologies suited for commercial production of both protein isolates (about 90% protein) and refined starch were developed. Wet-processing technologies were adapted to produce the initial ground legume slurry from which starch and fiber were removed by centrifugation. Starch was further purified using a series of liquid cyclones incorporating a countercurrent wash. Legume starches containing < 0.07% N were obtained in > 95% yield; they contained 32–35% amylose and, in a Brabender viscoamylogram, had restricted swelling characteristics typical of type C starches. Gels were rigid, opaque, and friable with a firmer texture than that of comparable corn gels; weeping losses through syneresis (13–15%) were prevented by acetylation (0.1 degree of substitution). Aqueous extraction of the legume seeds yielded approximately 94% recovery of total seed nitrogen; of this, 82% was recovered as an isoelectric-precipitated fraction (at pH 4.4) for an overall 77% recovery. An alternative protein purification process was also developed using ultrafiltration technologies. With these systems, an approximate overall 82% recovery of seed nitrogen (in the isolate form) was achieved. The protein isolates prepared by either isoelectric precipitation or ultrafiltration were spray-dried, and their functional properties were compared in terms of nitrogen solubility, foaming properties, water retention, color, flavor, and emulsification characteristics.

Researchers have made many efforts to develop processes using vegetable protein sources other than the soybean. Any alternative will be accepted only if its functionality and price are competitive with soybean-derived products. Such alternatives may be more desirable in countries that are net importers of vegetable protein and also have climates or population densities that make soybean production impractical.

Because field peas are already an accepted part of the human diet in many parts of the world, the components of the pea seed may be readily used by the food industry. Horsebeans, widely grown in parts of Europe and Asia, have also been suggested as an alternative source of human food protein (Flink and Christiansen 1973, Patel and Johnson 1974). Upgrading of the nutritional quality of horsebean protein by removal or destruction of toxic components (Lienert 1969) or indigestible carbohydrates (Cristofaro et al 1974) and the improvement of functional properties by processing may lead to increased acceptance of these legume proteins (Fan and Sosulski 1974). The details of process developments based on a modification of the classical wet processing of corn combined with technologies derived from the soybean isolate industry are discussed in this article.

MATERIALS AND METHODS

Air-dried, smooth-seeded yellow peas (Pisum sativum L. cv. Trapper) and horsebeans (Vicia faba equina L. cv. Diana) were provided by Woodstone Foods Ltd., Portage La Prairie, Manitoba, and by Northern Sales Ltd., Winnipeg, Manitoba. Seeds were first dehulled (giving 85 and 90% yield for horsebeans and field peas, respectively) with a plate mill followed by air aspiration.

Starch Preparation

The dehulled seeds (25 kg) were pin-milled (Alpine Pin Mill model 250 cw, Alpine American Corp., Natick, MA) and slurred with 0.03 N NaOH at a solid/liquid ratio of 1:4, w/w. The resultant slurry (pH 8.5) was screened (75-μm screen; Sweco Separator, Southwestern Eng. Co., Los Angeles, CA) to remove the fiber fraction and centrifuged (basket centrifuge, 32-cm diameter, operated at 2,400 rpm; Fletcher Works Inc., Philadelphia, PA) to recover the crude starch fraction. The supernatant fraction was then recentrifuged to remove both the fine fiber, mostly derived from the cell walls of the cotyledon (Schoch and Maywald 1968), and the dispersed protein. The starch centrifuge cake was reslurried with water (1:4, w/w) and recentrifuged. The starch thus obtained was spray-dried in a direct gas-fired spray dryer equipped with a cyclone collector (Proctor-Schwarz Corp., Philadelphia, PA) with an outlet temperature of 90°C.

An alternative scheme was developed for starch extraction and purification from the liquid extract. This involved the passage of the screened (75-μm) extract through a series of five hydrocyclone units (Dorr-Oliver Inc., Stanford, CT), each unit consisting of four nylon cyclones (each 1 × 10 cm). Their operation, shown in Fig. 1, involved the use of a countercurrent water wash system. The overs fraction from either the centrifuge or cyclones contained about 3–8% solids (60–70% protein) and was further purified to yield a protein isolate. This fraction was

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discharged through a cylindrical tube fixed in the center of the top and projecting some distance into the cyclone. The coarse fraction (starch) plus the remaining liquid was discharged from the cyclone through a small circular opening at the apex of the cone.

**Isolation of Protein by Isoelectric Precipitation**

This process involved acidification of the centrifuge or cyclone discharge to a fraction with 2N HCl to adjust the majority of the proteins to their isoelectric (IE) point at pH 4.4-4.6. The insolubilized protein curd was then collected by centrifugation (1,500 x g); the protein cake (about 85% protein) was resuspended in water (1:5, w/w) with the slurry maintained at pH 4.4. The precipitated protein was again recovered by centrifugation. The white cake (about 90% protein) was then diluted to 14% solids and 1 N NaOH was added to neutralize the slurry before it was spray-dried under the conditions indicated for the starch.

**Isolation of Protein by an Ultrafiltration Procedure**

The application of ultrafiltration (UF) technology was tested at the pilot plant scale using a Romicon hollow fiber cartridge (HF15-45-XM50) with a nominal molecular weight cut-off of 50,000 (Romicon Corp., Woburn, MA). The membrane system was comprised of a polysulfone composition incorporating 660 fibers, each of 0.114 cm id. The total ultrafilter area was 1.39 m². Operation was at 25 psig inlet pressure and 15 psig outlet pressure with a liquid extract process feed rate of 32 L/min in a closed loop. The initial flux through the membrane was 22.9 L per square meter per hour (L/m² h⁻¹). Operation was for 6-7 hr at 40-45°C. No prefiltration of the feed stream was required in any of the UF experiments.

An alternative UF system was also tested, using a tubular membrane unit with a 2.54 cm id (Abcor Inc., Cambridge, MA) coated with an HFM organic polymer (noncellulosic) with an average molecular weight cut-off of about 18,000. The Abcor model UF-44S pilot unit had a membrane area of 3.9 m² and was operated on a batch basis in a diafiltration mode. Feed rate was 273 L/min; water flux through this system was about 1,500 L/m² h⁻¹ at 25°C and 20 psig. For isolate production, inlet pressure was maintained at 50 psig and outlet pressure adjusted to 20 psig; operating temperature was 44°C. During diafiltration, water was mixed with the feed stream at a rate equal to the membrane flux. In a typical experiment at pH 8.0, 540 L of permeate was produced from an initial 310 L of feed from the hydrocyclones, the feed containing 2-3% solids (60-70% protein). Initial fluxes were 2,200 L/m² h⁻¹, decreasing to 1,200 L/m² h⁻¹ at the end of the experiment. Isolate production from a 60% protein feed stream required a 2-h UF period under these conditions.

A Dupont permeator model 0440-042 (Water Services of America Inc., Milwaukee, WI), operated at 150 psig, was used to further process the UF permeate stream by reverse osmosis.

**Analytical Methods**

The nitrogen content of the various products was determined by the standard AACC Kjeldahl method 46-10. Starch was analyzed by the dual enzyme, semimicro method of Banks et al. (1970). Amylograms were prepared on a Brabender Viscoamylo/Graph with a 700 cm-g sensitivity cartridge at 75 rpm, using AACC method 22-10. Acetylation of starches to a low degree of substitution was by Wurzburg's procedure (1964). Ash and moisture were analyzed by AACC methods 08-01 and 44-32.

Neutral and polar lipids were assayed by sequential Soxhlet extraction with hexane (AACC method 30-25) and then with chloroform/methanol (2:1, v/v). Neutral detergent fiber (insoluble dietary fiber) was determined according to AACC method 32-20. Cell wall polysaccharides were assayed by the sequential extraction method of Aspinall et al. (1966). Loss of birefringence was determined as described by Watson (1964) and amyllose by the method of Schoch (1964).

**Functional Properties of the Protein Isolates**

Nitrogen solubility index was determined by the standard AACC method 46-23. Water retention capacity was assayed by the procedure described by Solis (1972). Samples (5 g) were shaken for 1 hr with water (30 ml) at pH 7.0 and 25°C. Slurries were then centrifuged at 1,200 x g for 25 min and the supernatant liquid decanted. The weight of residue was used to calculate percentage water absorption.

Whipping (foaming) properties were studied using the procedure of Yasumatsu et al. (1972). Foam expansion was measured after 1 min at 25°C, foam stability after storage of samples for 30 min at 25°C. Vegetable protein suspensions (1%, w/v) were tested alone and in combination with skim milk powder and wheat flour (1:1, w/w). Samples were compared with foam properties of commercial soybean isolates (Promine F, Central Soya Co., Chicago, IL; Supro 620, Ralston Purina Co., St. Louis, MO).

Emulsifying activity was assayed by the procedure described by Yasumatsu et al. (1972). Corn oil (100 ml) and water (100 ml) were blended with the protein isolate (7 g) or with the isolate (5 g) plus wheat flour (5 g). The emulsifying ability of the isolate was also tested in NaCl solution (4%, w/v). All functional data are presented as averages of duplicate analyses.

**RESULTS AND DISCUSSION**

**Fractionation of Starch from Legumes**

The flow diagrams for the pilot-scale preparation of purified

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**Fig. 1.** Diagram of a multistage countercurrent hydrocyclone unit. 380 kPa gauge pressure.

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**Fig. 2.** Flow diagrams for pilot scale preparation of protein isolates and purified starch from field peas or horsebeans.
starch and protein isolates from field peas or horsebeans are presented in Fig. 2. Removal of lipid from these seeds before milling was not required; total lipid content (neutral plus polar lipids) of dehulled field pea and horsebeans seeds was 2.2–2.5%.

The protein contents of the dehulled seeds used in these experiments were 24–28% and 27–32% for the peas and horsebeans, respectively. Starch content varied from 44 to 48% in both sets of seeds.

Although repeated centifuging plus washing was found to produce high purity starches, the process did not lend itself to efficient pilot scale production. A preferred process that produced starch in maximum yield utilized hydrocyclones and a countercurrent water wash (Fig. 1) to remove and purify the starch.

An aqueous extract (13% total solids) from peas or horsebeans was fed to the No. 3 cyclone (Fig. 1). The resulting overs fraction contained 3.4% solids (containing only 0.8% of the original starch in the feed stream and 60.4% protein on a dry weight basis). The cyclone unders fraction contained about 99% of the starch. Even using the limited bank of five cyclones in series shown in Fig. 1, with feed entering at cyclone No. 3, 83% of the protein from the feed was partitioned into the overs fraction following a single pass through the apparatus. Three passes of the starch unders fraction resulted in more efficient separation and produced purified starch containing less than 0.07% nitrogen. The efficiency of the hydrocyclones for this process was based mainly on the large uniform size of the legume seed starch granules. For comparative purposes, corn starch slurries were also passed through this cyclone system with feed at cyclone No. 3; approximately 10% of the starch was lost in the overs fraction at cyclone No. 1. Further numbers of cyclones introduced into this series would serve to further purify the starch unders fraction, with a coincident overall saving of process wash water. The starch was obtained in over 95% yield from both field peas and horsebeans. This was equivalent to a 42–45% and 38–42% yield of starch from the dehulled field peas and horsebeans, respectively.

Legume starch slurries have higher viscosity values than do wheat or corn slurries of equivalent solids content (Vose 1977), a factor of relevance when planning commercial scale-up of these processes. Scanning electron micrographs showed irregular oval-shaped granules, often with deep superficial fissures. The granules were 20–40 μm in diameter, had a 98% birefringence end point at 70°C, and contained 32–35% amylose. The viscoamyllogram was typical of type C starches, exhibiting restricted swelling characteristics. Of particular interest was the stability shown when the starch was passed in acidic solutions (Comer and Fry 1978, Vose 1977). Horsebean starch had very similar properties to field pea starch, although a higher viscosity development occurred during pasting. Both starches exhibited considerable retrogradation on cooling of the gels; the gels thus obtained were rigid, opaque, and friable with a firmer texture than comparable corn starch gels. Gel shrinkage occurred following storage at 5°C, resulting in losses by syneresis of 13–15%; this phenomenon was prevented by acetylation to a low degree of substitution (0.1 DS).

### Legume Seed Fiber Components

The starchy unders fraction from the cyclones was further purified by passing it through a horizontal vibrating screen (45 μm). The screening removed some residual fiber that was presumably derived from the cotyledon cell walls (Schoch and Maywald 1968).

The fiber fraction from the dehulled seeds was subjected to a series of extractions (Aspinall et al. 1966) to identify the nature of these cell wall polysaccharides. In the case of the pea fiber, these were found to consist of 13% pectin, 25% lignin, 19% holocellulose soluble in ethylenediamine tetraacetic acid, and 13% α-cellulose.

(Extraction of the horsebean fiber yielded 38% pectin, 18% lignin, 11% holocellulose soluble in ethylenediamine tetraacetic acid, and 8% α-cellulose). This fiber fraction was strongly hygroscopic, readily absorbing 10–14 times its weight of water. It had a neutral detergent fiber content of about 30%, but only a trace of fiber as detected by the classical crude fiber procedure.

### Fractionation of Protein from Legumes

**Isoelectric Process.** The liquor from the centrifuge or cyclone overs, containing the dispersed protein from the dehulled seed, was processed by adjusting the pH to 4.4 with 2N HCl. The IE protein that precipitated out of solution was then removed by centrifugation, redispersed, and centrifuged to produce a protein isolate. The aqueous alkali (0.03 N NaOH, pH 8.5) extraction of the legume seed slurries resulted in 94% recovery of total seed nitrogen. Of this extract, 82% was recovered as an IE-precipitated fraction—an overall 77% recovery. These results are comparable to those reported by Fan and Sosulski (1974) and Hang et al. (1970).

**Ultrafiltration Process.** The other procedure developed for recovery of the protein in isolate form involved UF using noncellulosic types of membranes. Development of membrane systems stable over a wide pH range and at elevated temperatures has opened new opportunities for practical exploitation of this technology as an alternative to conventional acid-precipitation methods (Lawhon et al. 1978, Olson 1977). Removal of the low molecular weight components (soluble proteins, nonprotein nitrogen compounds, oligosaccharides, etc.) resulted in 88% recovery of protein from the feed stream. This represented an overall 82–83% recovery of protein in the isolate form from the dehulled grain legume seeds. The UF process using the hollow fiber system resulted in the concentration of a 4–5% solids feed (containing 60–65% protein) to a 12.8–13.5% solids retentate with an 89.5–94.1% protein content.

### Table 1

<table>
<thead>
<tr>
<th>Isolate and Source</th>
<th>Lipid Content, Extracted by</th>
<th>Ash (%)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Protein^a</td>
<td>Hexane</td>
</tr>
<tr>
<td>Isoelectric</td>
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<td></td>
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<tr>
<td>Pea</td>
<td>91.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Horsebean</td>
<td>91.2</td>
<td>0.9</td>
</tr>
<tr>
<td>Ultrafiltration</td>
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<tr>
<td>Pea</td>
<td>89.5</td>
<td>0.6</td>
</tr>
<tr>
<td>Horsebean</td>
<td>94.1</td>
<td>0.6</td>
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^aValues are an average of two determinations and are expressed on a dry weight basis.

N × 6.25.
The hydrocyclone over fraction was further clarified by centrifugation at 14 L/min through a Sharples ultracentrifuge at 1,200 × g. Centrifugation removed residual traces of starch and some fiber; the final feed to the ultrafiltrator contained 94–96% of the nitrogen from the dehulled seed. Dialfiltration was continued until the protein content of the feed stream solids reached 90% (N × 6.25), at which point the feed stream was concentrated to about 6% solids before pH adjustment to 6.8 and spray-drying. In this procedure, 85% of the nitrogen from the ultrafiltrator feed was recovered in isolate form, the balance being lost in the permeate. This represented an 80–82% total recovery of protein nitrogen.

The reverse osmosis unit (10-cm diameter) had a capacity sufficient to match the flux rate of the UF permeate stream; clear product water was achieved at a rate of 3 L/min (0.01–0.02% solids; product water resistance increasing from 5,000 to 9,500 Ω during the process). The reject stream was produced at a rate of 1.2 to 2 L/min (1.3% solids, decreasing to 0.25% during the processing of the UF permeate stream). No indication of decrease in reverse osmosis flux was seen during the process.

Data on the composition of the spray-dried protein isolates obtained by either IE precipitation or UF are presented in Table I.

Functional Properties of Protein Isolates

The data in Fig. 3 indicate that processing field peas and horsebeans to yield spray-dried protein isolates did not cause denaturation of the proteins, as would be evidenced by decreased solubility at acid or alkaline pH. The nitrogen solubility of the native seed protein (Vose et al 1976) is very similar to the spray-dried isolates (Fig. 3). The IE pea isolate had about 66% solubility at both pH 3 and 7; the results with the horsebean isolate (IE) were similar to but showed lower solubilities at pH 3.5 and 5.5 than did the pea isolate (IE). Isolates prepared by the UF procedure had higher solubilities in the IE pH range (15 and 22%, respectively, for the pea and horsebean UF isolates) than those prepared by IE precipitation. Also the point of minimum nitrogen solubility was at pH 5.0 rather than at pH 4.4, as observed with the IE isolates. In all cases, the products were readily dispersible in cold water. The protein isolates were fairly bland in flavor; pea isolates were a cream color, whereas the horsebean isolates were slightly darker.

The ability of these protein isolates to absorb water at pH 7.0 was also tested. The field pea (IE) and horsebean (IE and UF) samples absorbed 2.7–2.8 times their weight of water; the pea (UF) at pH 2.5 absorbed 3.3 times its weight of water, whereas the pea (UF) at pH 8.5 absorbed only 2.0 times its weight of water. These levels are lower than those generally observed with soybean isolates, which have water absorption capacity of 4–5 times their weight. The high solubility of these proteins may account for their lower apparent water absorption capacity (Quinn and Paton 1979).
The whipping (foaming) and emulsifying properties of these isolates were also studied (Fig. 4). The legume proteins were tested both alone and in combination with wheat flour and skim milk powder. The effect on emulsion stability of including 4% NaCl in this system was also studied. In 1% suspension, the horsebean (UF) isolate was superior to the other samples in foaming expansion and stability. The foaming abilities of all these isolates were compared with those of two commercial soybean isolates (Central Soya Promine F and Ralston Purina Supro 620) and found to be equivalent or superior. Blending the legume protein with skim milk powder significantly increased both foam stability and expansion characteristics, with the exception of the horsebean UF sample. Addition of wheat flour decreased the foam stability.

The emulsification activity of these isolates is shown in Fig. 5. Under the specific conditions used for this test, in which equal volumes of water and oil were emulsified with a sample of the protein isolate, the results obtained with the pea and bean IE and UF samples were comparable or lower to those obtained with Supro 620, when the emulsion was formed in a saline medium, the quality was equivalent to that of the Supro 620.

CONCLUSION

This work has demonstrated the feasibility of producing field pea and horsebean protein isolates with functionalities comparable to those of commercial soybean isolates. The other products of this process are an intermediate amylose starch, a hygroscopic fiber, a coarse hull fraction, and a whey effluent stream. An assessment of the economic potential for such a process development is complex and would depend on the marketability of the various fractions and particularly on the cost of disposal of the whey stream. The latter could be concentrated on a multi-effect evaporator and used for animal feed, or it could be further processed by application of ultrafiltration/reverse osmosis technologies to yield soluble protein (albumin), a soluble carbohydrate-rich fraction, and process water suitable for recycling in the process.

For those nations dependent on imported soybean meal or flour as a source of vegetable protein, the development of alternative crops is of considerable interest. The risks of future embargoes and shortages make dependence on a single supply source for such a vital commodity as vegetable protein unwise. In the future, the field pea and horsebean may play a role to fill this deficiency.

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LITERATURE CITED


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