Soy Protein Ingredients Prepared by New Processes – Aqueous Processing and Industrial Membrane Isolation

J.T. LAWHON, K.C. RHEE and E.W. LUSAS, Food Protein Research and Development, Texas A&M University, College Station, TX 77843

ABSTRACT

The production of food ingredients from undefatted soybeans by aqueous processing and isolation of protein from soy flour by ultrafiltration membranes has been demonstrated adequately during the past decade. These relatively new techniques offer significant advantages over conventional soy processing methods. Aqueous processing requires no petroleum-based solvent and consequently provides increased safety and flexibility of operation (because start-up and shutdown are safe and easy). It also provides opportunities for removal or deactivation of undesirable constituents of raw materials with appropriate water-soluble chemicals. It is, however, less efficient in oil extraction, and demulsification is required to recover clear oil when emulsions form. Ultrafiltration processes recover protein directly from soy flour extracts and thereby avoid generation of the whey which results from the conventional isoelectric precipitation. These processes have the advantages of increased isolate yield (as whey proteins are recovered in the isolate), and produce products having enhanced functionality and nitrogen solubility. The two processing techniques have subsequently been combined to obtain a single procedure with the advantages of each. Extracts from undefatted soybeans have been membrane processed with and without separating the oil to produce a variety of new soy protein ingredients.

INTRODUCTION

During the past three decades, new processes for producing oilseed protein products have been developed as alternatives to conventional procedures. Several processes have departed radically from standard extraction practices in that an aqueous system was employed to extract both oil and proteins (1-12). Two of these processes (5,7) are known to have been used commercially.

In other new and unconventional processes, ultrafiltration membranes have been employed to recover protein from defatted oilseed flour extracts as an alternative to isoelectric precipitation (13-22). Subsequently, a combination of aqueous processing and membrane processing was employed to produce a variety of oilseed products containing different ratios of protein and oil (23-28).

The Food Protein Research and Development Center (FPRDC), Texas A&M University, has developed processes embodying each of these three new processing strategies. The procedures and data resulting from FPRDC research will provide the primary basis for discussion in this paper.

AQUEOUS PROCESSING

Aqueous processing is a significant innovation in oilseed technology. As considered here, aqueous processing refers to processing undefatted oilseeds with water as the extracting solvent. It has been demonstrated that oil and protein can be removed simultaneously from oilseed materials by dispersing finely comminuted seed in water followed by centrifugal separation of the dispersion under suitably controlled conditions into oil, solid and aqueous phases. The bulk of the proteins may be recovered as concentrate in the solid phase or isolate in the aqueous phase, depending on the pH conditions selected. Protein may be recovered from the aqueous phase by isoelectric precipitation.

Unit operations in aqueous processing may vary for dif-

ferent applications, but as practiced in the FPRDC's Aqueous Extraction Process (AEP), consist of grinding, solidliquid separation, centrifugation, demulsification and drying (29).

To date, the AEP has been successfully applied to coconuts (10), peanuts (9,30,31), soybeans (12) and cottonseed (Rhee, unpublished data) and to a lesser degree to sunflower (32) and sesame seeds (33).

Advantages

Aqueous processing offers several significant advantages over conventional solvent extraction processes.

Safety. Because a flammable solvent is not used there is less danger of fires and explosions.

Pollution control. Solvent loss to the atmosphere is eliminated.

Discontinuous operation. Start-up and shutdown are safe and easy in the absence of a flammable solvent hazard, allowing the flexibility of discontinuous operation.

Capital investment. The smallest economic aqueous processing plant can be smaller than the smallest safe solvent extraction plant and would require less initial capital investment.

Extraction by water. No petroleum-based solvent is required to extract the oil or protein.

Detoxification. Appropriate water-soluble chemicals may be added to the aqueous system to remove or deactivate selected undesirable constituents in the raw material.

Disadvantages

Disadvantages inherent in aqueous processing include (a) slightly lower efficiency of oil extraction and recovery than by solvent extraction—the FPRDC AEP recovers only ca. 95% as much oil as conventional processes, (b) potential for reduced product stability because of higher oil content, (c) necessity for demulsification to recover clear oil when emulsions form, and (d) increased sanitation required to prevent microbial contamination.

Optimization of Processing Parameters

Application of aqueous processing to different oilseeds necessitates changing specific parameters to some extent because of the differing chemical compositions and physical structures of the seeds. In adapting the AEP to soybean processing, effects of particle size, solids-to-water ratio, extraction pH and extraction temperature on distribution of oil and protein in the isolate, residue and whey fractions were investigated. As expected, finer particle size gave more efficient extraction. More protein was recovered as isolate as particle size decreased; the oil content of isolate was reduced.

The optimum solids-to-water ratio was found to be 1:12 (w/w) and pH 9 was selected as optimal for extraction. Higher pH tended to recover more protein and oil in the isolate fraction. pH 9 also proved desirable for lipoxygenase inactivation. Extraction temperature was not a critical factor in protein recovery. Temperature was significant, however, in oil extraction. Maximal recovery occurred in the temperature range of 40 to 60 C. Consequently, after consideration of the effect of temperature on lipoxygenase inactivation, an extraction temperature of 60 C was found optimal. A 30min extraction period was sufficient for extracting both oil and protein, and a protein precipitation pH of 4.5 yielded maximal recovery of both oil and protein.

The AEP as adapted for soybean processing consisted of preconditioning cleaned beans by heating at 70 C to achieve a 6% moisture content, to facilitate grinding and reduce enzyme activity. Beans were dehulled by cracking and aspirating the hulls. A Contraplex pin mill was used to reduce particle size (99% of which was <70 mesh, U.S. Sieve Series, and 85% of which was <100 mesh).

Ground beans were then extracted at 60 C with water containing 0.01% of hydrogen peroxide for lipoxygenase inactivation. A solids-to-water ratio of 1:12 by weight was employed in a 30-min extraction at pH 9. The aqueous slurry was centrifuged to separate an aqueous phase, a solids phase and an oil/emulsion phase.

The solids phase, although primarily insolubles, also included some recoverable protein and oil, and was further treated by resuspension and washing at a solids-to-water ratio of 1:5 with the pH maintained at pH 9. Upon recentrifugation, the resulting fractions were combined with those from the initial centrifugation.

The combined aqueous phases were adjusted to pH 4.5 with HCl to precipitate the protein. The protein curd was separated by centrifugation and spray-dried to yield a protein isolate. Washing the curd before drying raised its protein content. The solids phase, a fibrous residue with potential use as a livestock feed, was simply dried. The combined oil/ emulsion phases were further processed to recover clear oil.

Table I shows optimal conditions for demulsification of the oil. Moisture level proved to be the most critical factor in demulsification. Allowable moisture was found to range from 20-23%. This was obtained by addition of soybean oil to the emulsion. Other important requirements as shown in Table I were pH of the emulsion, mode of agitation (a shear-

TABLE I

Optimum Conditions for Demulsification

Parameter	Condition
Allowable moisture level	20% with a maximum of 23%
pH	4.5 with a range of 4–6
Mode of agitation	Shearing action
Speed of agitation	Slow
Length of agitation	1–3 min
Speed of centrifuge	1,000 rpm or higher
Length of centrifugation	1–3 min
Temperature	40 C or higher

TABLE II

Proximate Analysis of AEP Soy Isolates

Isolate treatments	Moisture	Protein (Nx6.25)	Oil	Total sugars	Crude fiber	Ash
			% Dry	v wt basis		
No wash steps	5 1.6	82.4	7.4	5.5	0.8	2.3
Residue washing only	1.3	81.8	8.3	5.4	1.4	1.8
Protein curd washing only	1.6	89.6	3.2	3.6	0.4	1.6
Residue and curd washings	1.5	89.2	3.6	3.1	0.6	2.0



FIG. 1. Simplified flow diagram of the Aqueous Extraction Process applied to soybeans.

ing action was needed), speed of agitation, duration of agitation, centrifuge speed and duration of centrifugation.

Product Characteristics

Oil. The AEP oil had peroxide values of 0.6-0.9 meq/kg oil, free fatty acid (FFA) value of 0.5-0.7% and a refining loss of 1.2-2.0%. Corresponding values for oil extracted with hexane from the same lot of soybeans were 1.7-1.9 PV, 1.8-2.0% FFA and 4.1-4.9% refining loss.

Protein isolaté. Table II contains analytical data on AEP protein isolates from procedures employing different combinations of wash steps. By washing the protein curd with acid water, the protein content of dry isolate was increased to almost 90% on a dry basis and its oil content decreased to ca. 3%. However, the amount of isolate recovered was decreased by 1.5%.

Heat treatment at 60 C reduced enzyme activity considerably (from 100 to 22 lipoxygenase units/mg ground soy at pH 4.5 and to 2 units at pH 9.0). The remaining lipoxygenase activity can be effectively stopped by addition of 0.01% hydrogen peroxide at pH 9 or 0.03% at pH 4.5.

Investigations at the FPRDC demonstrated that the aqueous processing of soybeans was technologically feasible. Nevertheless, the process needs to be improved in areas such as the protein content of the isolate and the oil yields. Figure 1 is a simplified flow diagram of the soybean AEP.

MEMBRANE ISOLATION

Advances in Protein Isolation

The conventional method of isolating protein from defatted oilseed flours generates a whey-like liquid which poses serious disposal problems and represents a significant loss of edible protein and other valuable constituents. During the last decade, this undesirable feature motivated the study of membrane processing of protein extracts as an alternative method of protein recovery.

In 1970, Michaels and Porter (13) reported success in concentrating and purifying protein from soybean meal. Fraseur and Huston (14) patented a process to recover protein from defatted soybeans by preparing a slurry, homogenizing the slurry, clarifying it by centrifugation, and then processing it by UF. Iacobucci et al. (15) patented an isolation process to produce isolates having low phytic acid content by ultrafiltering protein extracts in the presence of suitable chemical reagents. Chemical treatment involved either enzymatic hydrolysis of phytic acid by the enzyme phytase prior to UF or UF in the presence of calcium ion at low pH, or the presence of a strong chelating agent such as ethylenediaminetetraacetic acid when processing in the pH range of ca. 7.0-11.0. Goodnight et al. (17) also patented a process employing UF which claimed to produce soy protein having low phytic acid content, improved digestibility, high water solubility, improved functional characteristics and absence of beany flavor with improved palatability and nutrition. Phytic acid was removed in the process by extraction of defatted soybeans and separation of insoluble materials at a pH in excess of 10.1. UF of the clarified extract at a pH below 10 was then performed with an optional heat treatment prior to UF.

FPRDC UF and RO Research

Research with industrial membrane systems began at the FPRDC in 1971. UF membranes were used to recover protein from cottonseed wheys (33). RO membranes were used to process UF permeate. The feasibility of recycling the effluent from RO membranes was demonstrated (34), indicating the potential for a "zero discharge" process. With the advent of noncellulosic, "second generation"

With the advent of noncellulosic, "second generation" UF membranes in 1975, FPRDC researchers began ultrafiltering oilseed flour extracts directly, avoiding the creation of by-product wheys (18). Eight UF and five RO systems were ultimately evaluated for processing oilseed protein extracts (35). Figure 2 is a simplified flow diagram for soy protein isolation with the FPRDC's MIP.

Extract preparation. Protein extracts were prepared by extracting 50-80-lb quantities of a high nitrogen solubility soy flour (Central Soya's Soy Fluff 200W) and a partially toasted soy flour supplied by A.E. Staley Mfg. Co. (NSI = 56). Either two extractions (10:1 water-to-flour ratio followed by 8:1) or a single extraction (30:1 ratio) were made with tap water adjusted to pH 9 or 8. The relative effectiveness of sodium hydroxide and calcium hydroxide for solubilizing soy solids and nitrogen were compared at pH9(19). Yields of solids and nitrogen in Ca(OH)₂ extract were observed to be as high or higher than those achieved with NaOH. Hence, Ca(OH)₂ became the hydroxide of choice in subsequent extractions. An extraction temperature of 43 C was used with high nitrogen solubility flour, and 55 C with toasted flour. Extraction continued for 40 min before removing insolubles from the slurry by centrifugation.

If two extractions were made, insoluble residue was resuspended in tap water at the same pH and temperature for an additional 20 min. Liquid supernatants were then pasteurized by heating to 63 C for 30 min. (This pasteurization step would be unnecessary in a continuous commercial operation).

Membrane processing technique. After pasteurization and prefiltering, feed solutions were ultrafiltered. Extract was processed in volumes up to 250 gal. Either a dilution technique applied after a 4.5:1 reduction in original feed volume or a diafiltration technique was employed to purify the protein retentate.

Dilution consisted of adding to the concentrated feed a quantity of filtered water equal to three times its volume



FIG. 2. Simplified flow diagram for soybean protein isolation with UF and RO membranes.

and reconcentrating it. Diafiltration consisted of adding filtered tap water to the extract during concentration at the same rate at which UF permeate was being removed. Diafiltration was begun after the initial extract volume was reduced by 60% and was continued until the total volume of UF permeate recovered at the end of the run equalled one and one-half times the initial extract volume. Feed temperature was maintained at 65 C throughout to give increased flux and prevent microbial build-up.

Product characteristics. Proximate analyses of MIP soy isolates from toasted and untoasted soy flours and a commercial soy isolate are compared in Table III. The MIP isolates were extracted using $Ca(OH)_2$. They contained 7-10 times as much calcium as NaOH-extracted commercial isolates and ca. one-half as much sodium. The use of Ca(OH)₂ has added significance, as sodium is increasingly linked to hypertension. Color values shown are L-scale readings from a Hunter Digital Color and Color-Difference Meter. Measurements were made on products as a powder and then as a wet paste. Higher readings indicate lighter color.

Nitrogen solubility profiles and NSI values on MIP isolates and a commercial isolate are compared in Table IV. The membrane-produced products are clearly superior in this property. Data on other functional properties of MIP products were also compared with corresponding measurements on a commercial isolate and found to be equal or superior in all instances (36). Utilization tests have been conducted in which membrane-produced soy isolates were used to partially replace nonfat dry milk solids in soft-serve frozen desserts (37) and for protein fortification of wheat bread (38).

Economic analysis. An economic analysis was performed on three sizes of hypothetical soy MIP plants (39). Plants de-

TABLE III

Proximate Analysis of MIP Soy	Isolates from Untoasted and Toasted Flour and a Commercial Soy	/ Isolate

Product description		Nitr	Nitrogen		Total	Total			Color		
	Ash	Total	NPN	(Nx6.25)	P	sugars	Ca	Na	Dry	Wet	
MID in last					% Dry wt b	asis					
MIP isolate (untoasted flour)	6.3	6.3	14.76	0.25	92.3	1.2	5.5	0.93	0.54	84.5	66.8
MIP isolate (toasted flour)	7.5	14.68	0.33	91.8	1.2	5.8	1.3	0.60	82.4	61.6	
Commercial isolate	3.8	14.70	0.21	91.8	0.6	4.1	0.13	1.20	82.5	66.3	

Percentage of Soluble Nitrogen in I	MIP Soy Isolates from Untoasted	and Toasted Flour and a Commercial Soy Isolate
-------------------------------------	---------------------------------	--

Product	pH of measurement											
description	NSI	2	2.5	3	3.5	4	5.5	6	7	9		
MIP isolate (untoasted flour)	100.0	98.3	100.0	100.0	19.9	7.4	11.3	100.0	100.0	100.0		
MIP isolate (toasted flour)	95.7	97.7	94.8	90.6	12.4	5.1	8.2	87.7	91.8	94.1		
Commercial isolate	67.3	66.2	59.8	47.0	38.3	6.6	26.5	45.1	66.1	69.7		

TABLE V

Required MIP Soy Isolate Selling Prices for Various DCFRR^a

	Annual plant isolate production (MM lb)							
(%)	5	15	20					
0	0.67	0.52	0.50					
10	0.83	0.63	0.60					
20	1.02	0.76	0.73					
30	1.24	0.92	0.87					

^aCorresponding selling prices for commercial soy isolates ranged from 77-92¢/lb at time of study.

^bDiscounted cash flow rate of return.

signed to produce 5, 15 and 25 million lb of isolate/year were studied. Profitability of the three plants was evaluated by the discounted-cash-flow rate-of-return (DCFRR) method. This method of venture analysis gives the rate of return on investment after income taxes. Table V shows the required MIP soy isolate selling prices for various DCFRR. The MIP applied to soy was calculated to be economically profitable. Greatly improved isolate yields resulted from inclusion of whey proteins in the isolate. The distribution of soy flour solids and nitrogen during isolation for the MIP and conventional process are compared in Figure 3. Over 40% more isolate is obtained from the MIP.

The data of Table VI demonstrate the suitability of the RO effluent for reuse as process water. RO effluent was lower in total solids than local tap water employed in the extraction step. Conductivity and COD measurements on the RO permeate also are given.

AQUEOUS EXTRACTION AND MEMBRANE ISOLATION COMBINED

Aqueous processing and membrane processing have been combined in several effective ways. Goodnight, Jr., et al. (26) extracted ground whole soybeans at a pH of 7-9 to obtain a soybean lipid protein extract which, after clarification, was ultrafiltered to remove the carbohydrates. It was discovered that the presence of suspended or emulsified fat



FIG. 3. Distribution of soy flour solids and nitrogen for membrane and conventional isolation processes.

in the extract did not interfere with the efficiency of UF processing, and that the fat remained in the retentate.

When desiring to eliminate phytic acid from the full-fat product, the extraction was conducted in the pH range of 10.1-14 to render the phytates insoluble. Phytates were then separated along with other insoluble constituents by centrifugation. Subsequently, the extract was UF processed to remove carbohydrates. Up to 80% of the ground bean protein was recovered in a full-fat product containing as little as 0.002 g phytic acid/100 g protein.

Omosaiye et al. (24) used a hollow-fiber UF system to produce a full-fat soya protein product from whole soybean extract while simultaneously removing undesirable oligosaccharides. They found the amount of residual oligosaccharides to be a function of the amount of permeate removed. Products containing 59.5% protein, 34.2% fat and as little as 0.6% oligosaccharides were membrane produced. Omosaiye et al. (25) later reported success in removing phytic acid from a ground soybean extract by membrane processing. UF at pH 6.7 followed by dilution and reultrafiltration at the same pH eliminated ca. 92% of the phytic acid. A final product containing 60% protein, 35% fat and 0.064 g phytic acid/100 g solids was produced.

AEP-MIP Combination at the FPRDC

At the FPRDC, the AEP and MIP were combined in 1978 into a single procedure with the several advantages of each. The AEP-MIP combination was used to process three lots of soybeans. One lot was a composite of varieties, another consisted of Bragg variety beans and the third was White Hylum (Amsoy 71) beans. Three types of products (low-fat, intermediate-fat and full-fat) were prepared using alternative modes of operating the AEP-MIP.

Extraction procedures. Fifty-lb lots of undefatted soybeans were ground in an Alpine Contraplex pin mill to a particle size of ca. 100 mesh and extracted with prefiltered tap water. Water-to-bean ratios ranging from 30:1 to 12:1 by weight were tested in single extractions. Hydrogen peroxide at the level of 0.01% by weight was added to the extracting water

TABLE VI

Data on RO Effluent from Processing Soy UF Permeate

Solids	RO feed	1.24
content	RO effluent	0.029
(%)	Local tap water	0.055
Conductivity	RO feed	2800 @ 27 C
measurements	RO effluent	209 @ 27 C
(µmhos)	Local tap water	785 @ 27 C
COD measurements (mg/l)	RO feed RO effluent	10,673. 203.6

in about half of the extractions to assess its effect on lipoxygenase activity in finished products.

Extractions were conducted at pH 9, 8 and 6.65 (the pH of tap-water-ground bean slurry) for 30 min at 60 C. Ca(OH)₂ and NaOH were used in adjusting slurries to alkaline pH.² Upon completion of extraction, insoluble residue was separated by two-phase centrifugation, leaving a full-fat extract containing oil, protein and water. The full-fat extract was then either (a) ultrafiltered directly to produce a full-fat product, (b) subjected to three-phase centrifugation to separate oil from protein and water and produce a low-fat extract for UF processing or, (c) ultrafiltered directly to increase oil concentration in the extract prior to three-phase centrifuga-



FIG. 4. Simplified flow diagram for production of a full-fat product from undefatted soybeans with UF and RO membranes.







FIG. 6. Simplified flow diagram for production of an intermediatefat product from undefatted soybeans with UF and RO membranes.

tion to produce a low-fat protein fraction for spray drying. Simplified flow diagrams of these procedures are shown in Figures 4, 5 and 6.

Membrane processing techniques. Each type extract was processed by the tubular UF system of Abcor, Inc. The UF unit contained 22 ft^2 of either HFM 100 or HFM 180 non-cellulosic membrane.

After pasteurization and prefiltering, extracts were processed in volumes of 50-150 gal. Feed temperature was maintained at 65 C and the pH at 6.8-7.0. Either a dilution technique applied after a 4:1 volume reduction in original feed, or a diafiltration technique was used to purify the protein retentate.

UF permeate from low-fat extract was processed with a tubular RO system manufactured by Western Dynetics, Inc. The system was equipped with cellulose-based membranes having a rejection for 5,000 ppm NaCl of 95% at 500 psi. Operating temperatures were restricted to below 49 C and feed pH was maintained at 7.

Solids and nitrogen distributions. Distributions of soybean solids and nitrogen during processing by the procedures of Figures 4, 5 and 6 are given in Figure 7. In the full-fat process, 68.1% of ground bean solids and 85.7% of the nitrogen were recovered in full-fat product (FFP). The RO product contained 7.7% of the bean solids and 3.6% of the nitrogen. Insoluble residue retained 24.2% and 10.7% of bean solids and nitrogen, respectively.

Solids in the FFP include the oil. These trials demonstrated that industrial UF systems can effectively process high-oil feeds.

Low-fat product (LFP) contained 47.4% of ground bean solids and 76.9% of the nitrogen. Insoluble residue contained 28.8% of the solids and 15.3% of the nitrogen. Increased percentages of residual nitrogen and solids over those of the full-fat process resulted from the addition of insolubles removed during three-phase centrifugation to those separated in the initial centrifugation. Oil emulsion from the threephase separator contained 14.8% of starting solids and 5% of starting nitrogen. Perhaps a more efficient centrifuge would have left less nitrogen in the oil fraction.

The third procedure, the Intermediate-Fat Process, was derived in an attempt to achieve a more efficient oil separation by increasing the oil concentration in the feed before centrifugation. The intermediate fat product (IFP) contained 55.1% and 84.6% of the bean solids and nitrogen, respec-



FIG. 7. Distribution of soybean solids and nitrogen from aqueous extraction and membrane processing.

Extract	Water-to-bean	Abcor	Mean flux	Mean retentions (%)						
type	ratio	membranes	(gfd)	Solids	Nitrogen	NPN	Ash	Sugars		
D.U.C.	30 to 1	HFM-180	30.6	90.1	95.7	39.3	59.6	50.6		
Full-fat	30 to 1	HFM-100	42.6	89.0	96.7	43.2	64.2	47.8		
	25 to 1	HFM-180	29.4	85.7	96.6	48.6	50.6	51.1		
Low-fat	12 to 1	HFM-180	20.0	83.1	96.3			38.5		

Summary of UF Membrane Performance on Full-Fat and Low-Fat Extract from Ground Soybeans

tively. The RO product and residue product from this process were the same as from the Full-Fat Process. The oil emulsion fraction from the Intermediate-Fat Process contained 13% of the bean solids and 1.1% of the nitrogen. The oil emulsion fraction contained less nitrogen as anticipated (1.1% instead of 5.0% for the Low-Fat Process). However, more oil was retained in the IFP than the LFP as will be apparent in the product data to be discussed later. One advantage envisioned for the Intermediate-Fat Process was that higher water ratios could be used with it because oil concentration is increased prior to the second centrifugation. Thus, the benefits of increased nitrogen extractability could be obtained without affecting three-phase separation efficiency.

TABLE VIII

TABLE VII

Performance of a Tubular RO System on UF Permeate from Low-Fat Soybean Extract

	Performance measurement	s
Mean flux	18.5	
Solids content (%)	RO feed RO effluent Local tap water	0.740 0.012 0.055
Conductivity measurements (µhmos)	RO feed RO effluent Local tap water	1600 71.6 760

TABLE IX

Analytical Data on Full-Fat, Intermediate-Fat and Low-Fat AEP-MIP Products

Membrane performance. UF membrane performance on fullfat and low-fat extracts is depicted in Table VII. Mean flux was higher when extracts were prepared with higher water ratios. Of the two membranes used, the HFM-100 membrane gave higher flux. The HFM-100 membrane has a theoretical molecular weight cut-off of 10,000. The HFM-180 has a cutoff point of 18,000. Mean percentage retentions of various feed components also are shown in Table VII.

Table VIII shows performance data obtained from RO processing of UF permeate. The RO effluent was again lower in total solids than local tap water, indicating its suitability for reuse without further treatment. Conductivity measurements on RO permeate were also lower than those of local tap water. A mean flux of 18.5 gal/ft² of membrane area/ day (gfd) were obtained during processing.

Product characteristics. Analytical data for each type product are presented in Table IX. Oil contents shown were determined by ether extraction. Results from efforts to measure oil unextractable by ether were inconsistent. However, data obtained on LFP indicated they contained from 4 to 8% oil in a bound form. Higher oil contents in products resulted in lower protein. Ash and sugar contents were, in general, acceptably low. The levels of these components can be controlled by altering the dilution technique. Desirable high NSI values were obtained in each type product. Trypsin inhibitor and urease activity values for the IFP were unexpectedly high. A probable cause for this is now known. However, the several IFP trypsin and urease measurements made were consistent.

Product type Nitrogen Total Protein NPN Total (Nx6.25) Total Ash Total sugars Crude fiber Oil Ether ext'n. Trypsin inhibitors Urease urease % Dry wt. basis	Nitrogen		Protein		Total	Crude	Oil Ether		Trypsin inhibitors	Urease	Color	
	Dry	Wet										
			%]	Dry wt.	basis							
Low-fat	11.81	0.31	73.82	4.2	7.5	0.07	1.9	100.0	26.3	0.02	80.2	61.8
Intermediate fat	12.63	0.21	78.92	3.5	4.8		9.8	95.9	71.2	1.79	84.4	75.3
Full-fat	10.57	0.37	66.04	3.9	5.5	0.08	32.3	100.0	25.8	0.06	81.8	73.4
Residue product	3.07	0.27	19.17	3.9	2.3	18.4	12.3	56.3			86.3	71.0

TABLE X

Nitrogen Solubility Profiles on Low-Fat, Intermediate-Fat and Full-Fat AEP-MIP Products

Product	pH of measurement										
type	2.0	2.5	3.0	3.5	4.0	5.5	6.0	7.0	9.0		
Low-fat	83.8	86.7	86.5	58.6	9.9	8.7	52.6	90.9	96.7		
Intermediate-fat	96.3	94.7	89.2	85.0	10.7	9.9	71.5	81.9	96.9		
Full-fat	84.1	92.2	85.1	88.4	6.6	7.4	40.6	52.0	96.8		

Each of the L-scale color values on the products were satisfactorily high. The lighter color of the IFP is attributed to its preparation from White Hylum beans. Data on the insoluble residue product are also shown in Table IX.

Nitrogen solubility profiles prepared by measuring solubilities at 9 pH are given in Table X. Solubilities were generally high below pH 3.5 and above pH 7.

The essential amino acids and available lysine for each type product are shown in Table XI. Some of the variation among products is attributable to varietal differences. The IFP and FFP were prepared from a different variety of beans than those used for the LFP, as indicated.

Product stability in storage. Full- and low-fat products were stored at three temperatures (room, 60 and 100 C) and lipid oxidation measured initially, after 1 week and after two weeks of storage (with the exception that products stored at 100 C were not measured after two weeks).

Lipid oxidation measurements are given in Table XII. TBA number for the LFP was initially lower than for the FFP. TBA numbers showed little change over a two-week period at room temperature for either type product. However, at 60 C, TBA number of the FFP had risen from 2.5 to 31.0 after one week and to 69.7 after two weeks, which is nearly 30 times as high as the initial TBA value. A similar TBA value increase was found in the FFP after one week at 100 C; it was not measured at the end of two weeks. The LFP registered much less change in TBA number than the FFP at 60 C as well as 100 C.

Based on these TBA tests and the lipoxygenase determinations which follow, it was concluded that the extent of lipid oxidation in AEP-MIP products prepared with H_2O_2 treatment is dependent on the substrate (oil) concentration and the temperature and length of storage.

Lipoxygenase activity in products. Data from lipoxygenase measurements on products are presented in Table XIII. The activity of purified lipoxygenase was also determined for reference and comparison. Undefatted, ground soybean samples, whether unheated or heated at 100 C for 1 hr, had high enzyme activity. It was apparent that the 1-hr heat treatment at 100 C was insufficient to inactivate the enzyme in the crude product. No activity was measurable in any of the other samples analyzed which included full-fat and low-fat products prepared at varying extraction pH and with and without H₂O₂ treatment in extraction.

with and without H_2O_2 treatment in extraction. Relative to the LFP sample prepared with H_2O_2 omitted from the extracting solvent, the extraction temperature (60 C) and the length of extraction at that temperature (30-40 min) plus pasteurization and membrane processing at 65 C may have been enough heat treatment to inactivate the lipoxygenase without H_2O_2 -even at pH 7.0, which is a favorable pH condition for lipoxygenase activity.

Analyses of the RO product from processing UF permeate are given in Table XIV. It contained about one-half sugar and one-fifth ash, and 11.5% protein. Potential applications for this product were not investigated. Compositional data on RO feed and effluent streams also are given in Table XIV.

Oil recovery. Material balance data reflecting oil distribution and recovery in the Low-Fat and Full-Fat Processes were not taken. Data were already available from previous FPRDC investigations using the AEP which showed ca. 86% of the oil in the beans to be recoverable as clear oil. Processing procedures in that work were identical with those of the AEP-MIP through the oil separation step.

In the Intermediate-Fat Process, oil recovery is reduced by the difference in oil between the IFP and LFP. In the Full-Fat Process, of course, the oil is recovered in the FFP.

Economic analysis. The economics of using the AEP-MIP

TABLE XI

Essential Amino Acid Composition of Low-Fat, Intermediate-Fat and Full-Fat AEP-MIP Soy Products

	Low-fat (Composite of var.)	Intermediate-far (White Hylum var.)	t Full-fat (White Hylum var.)
Amino acids	Protein (%)	Protein (%)	Protein (%)
Lysine	6.1	6.4	6.6
Tryptophan	1.8	1.5	1.4
Threonine	3.9	4.1	4.1
Valine	4.9	4.9	5.0
Methionine	1.3	1.2	1.0
Isoleucine	4.8	4.8	4.9
Leucine	8.5	8.0	8.2
Phenylalanine	5.5	5.3	5.4
Available lysine	5.9	6.1	6.2

TABLE XII

Lipid Oxidation in Full-Fat and Low-Fat AEP-MIP Soy Products before and after Storage

Product description	Storage temperature	TBA number (mg malonaldehyde/kg sample)			
		0 days	1 week	2 weeks	
Full-fat	Room (C) 60 100	2.5 2.5 2.5	2.9 31.0 28.9	3.2 69.7	
Low-fat	Room (C) 60 100	1.5 1.5 1.5	2.1 6.0 14.8	2.4 15.4 	

TABLE XIII

Summary of Lipoxygenase Activity Measurements on AEP-MIP Products

Samp analy:	le zed (Increas	Lipoxygenase activity e of absorbance at 234 m/min/g)
Purified soybean	lipoxygenase	2913.3
Undefatted groun	d soybeans	429.2
Undefatted groun (heated @ 100 C :	d soybeans for 1 hr)	412.5
Low-fat product, (0.01% H ₂ O ₂ in e	pH 7 extraction)	No activity
Full-fat product, (0.01% H ₂ O ₂ in e	pH 7 extraction)	No activity
Low-fat product, (H ₂ O ₂ omitted fr	pH 7 om extraction)	No activity

to produce intermediate- and full-fat products were assessed (40). Materials balance and product quality data from FPRDC pilot plant runs were used in the analysis of hypothetical plants sized to process 28.14 million lb of whole soybeans annually. Profitability was appraised by performing a DCFRR analysis on two plants, i.e., a plant to produce IFP and a plant to produce FFP. The analyses were prepared for three levels of return-10, 20 and 30%. The required concentrate selling price to yield these rates of return are shown in Table XV. The required selling price at 0% DCFRR also was calculated. These prices include all capital and operating costs associated with production of the concentrates. An allowance for marketing and research and development costs would be incurred, however, and should be considered when determining a final sales price.

TABLE XIV

Analytical Data on RO Product from Processing UF Permeate from Low-Fat Soybean Extract

Fraction Solid	Solids	Nitrogen (%)		Protein (Nx6 25)	Ash	Sugar	
analyzed	(%)	Total	NPN	(%)	(%)	(%)	
Feed to RO membrane	0.47	0.09 (1.84) ^a	$0.007 \\ (0.15)^{a}$	0.54 (11.53) ^a	0.93 (19.9) ^a	0.24 (5.1) ^a	
RO concentrate (spray-dried)	91.65	1.84 (2.01)	1.2 (1.3)	11.53 (12.58)	19.9 (21.7)	50.5 (55.1)	
RO permeate	0.012	0.0003 (2.62)		0.002 (16.42)	0.01 (80.7)		

^aDry weight basis.

TABLE XV

Concentrate Selling Price at Various Discount-Cash-Flow Rates of Return (DCFRRS)

DCFRR (%)	Concentrate selling price (\$/lb)		
	Intermfat	Full-fat	
0	0.55	0.48	
10	0.74	0.63	
20	0.96	0.80	
30	1.22	1.01	

Selling prices for these products cannot be directly compared with commercial soy products currently available because AEP-MIP products contain more fat. The fat in these concentrates could increase their desirability in appropriate applications. A break-even selling price (0% DCFRR) was calculated to be \$0.55/lb for the IFP and \$0.48/lb for the FFP as of September 1979.

The apparent economic feasibility of the AEP, MIP, and AEP-MIP along with their several significant advantages make them viable and attractive options for producing soya protein food ingredients.

ACKNOWLEDGMENTS

The FPRDC research described herein was funded by the Natural Fibers and Food Protein Commission of Texas, the Andersons, Maumee, Ohio, the National Science Foundation and Kraft, Inc., Glenview, IL.

REFERENCES

- 1. Chayen, I.H., and D.R. Ashworth, J. Appl. Chem. 3:529 (1953).
- 2. Sugarman, N., U.S. Patent 2,762,820 (1956).
- 3. Dangoumau, A., Rev. Fr. Corps Gras 5:254 (1958).
- Subrahmanyan, J., D.S. Bhatia, S.S. Kalbag and N. Subramanian, JAOCS 36:66 (1959).
- 5. Chayen, I.H., U.S. Patent 2,928,821 (1960).
- Rajasekharan, N., "Chemical and Technological Investigations on Coconut Products," Thesis, Banaras Hindu University, Utter Pradash, India, 1964.
- 7. Bhatia, D.S., M.A.B. Parpia and B.P. Baliga, J. Food Sci. Tech-

nol. (India) 2:3 (1966).

- Eapan, K.E., S.S. Kalbag and V. Subrahmanyan, J. Am. Chem. Soc. 43:585 (1966).
- 9. Rhee, K.C., C.M. Cater and K.F. Mattil, J. Food Sci. 37:90 (1972).
- 10. Hagenmaier, R.D., C.M. Cater and K.F. Mattil, Ibid. 38:516 (1973).
- 11. Oberg, E.B., U.S. Patent 4,075,361 (1978).
- 12. Mattil, K.F., K.C. Rhee and C.M. Cater, U.S. Patent 4,151,310 (1979).
- 13. Michaels, A.S., and M.C. Porter, Applications of Membrane Ultrafiltration to Food Processing, presented at the 3rd International Congress of Food Science & Technology, Washington, DC, Aug. 1970.
- 14. Frazeur, D.R., and R.B. Huston, U.S. Patent 3,728,327 (1973).
- 15. Iacobucci, G.A., D.V. Myers and K. Okubo, U.S. Patent 3,736,147 (1973).
- Pompei, C., S. Maletto and M. Lucisano, Production D'isolats De Soja Par Ultrafiltration Et Diafiltration, presented at the 4th International Congress of Food Science & Technology, Sept. 1974.
- 17. Goodnight, K.C., Jr., G.H. Hartman, Jr., and R.F. Marquardt, U.S. Patent 3,995,071 (1976).
- Lawhon, J.T., D. Mulsow, C.M. Cater and K.F. Mattil, J. Food Sci. 42:389 (1977).
- 19. Lawhon, J.T., D.W. Hensley, M. Mizukoshi and D. Mulsow, Ibid. 44:213 (1979).
- 20. Lawhon, J.T., L.J. Manak and E.W. Lusas, Ibid. 45:197 (1980).
- 21. Nichols, D.J., M.S. Thesis, Univ. of Illinois, Urbana, 1979.
- 22. Nichols, D.J., and M. Cheryan, "Production of Soy Isolates by Ultrafiltration," Presented at the 40th Annual Mtg. of Inst. of Food Tech., New Orleans, LA, June 1980.
- 23. Cheryan, M., and J.E. Schlesser, Lebensm. Wiss. Technol. 11: 65 (1978).
- Omosaiye, O., M. Cheryan and M.E. Matthews, J. Food Sci. 43:354 (1978).
- 25. Omosaiye, O., and M. Cheryan, Ibid. 44:1027 (1979).
- Goodnight, K.C., Jr., and G.H. Hartman, Jr., U.S. Patent 4,088,795 (1978).
- 27. Lawhon, J.T., L.J. Manak, K.C. Rhee, K.S. Rhee and E.W. Lusas, "Combining Aqueous Extraction and Membrane Isolation Techniques to Recover Protein and Oil From Soybeans," submitted for publication in J. Food Sci.
- Lawhon, J.T., L.J. Manak, K.C. Rhee and E.W. Lusas, "Production of Oil and Protein Food Products from Raw Peanuts by Aqueous Extraction and Ultrafiltration," submitted for publication in J. Food Sci.
- Cater, C.M., K.C. Rhee, R.D. Hagenmaier and K.F. Mattil, JAOCS 51:137 (1974).
- Rhee, K.C., C.M. Cater and K.F. Mattil, Cereal Chem. 50:395 (1973).
- Rhee, K.C., K.R. Nataragan, C.M. Cater and K.F. Mattil, JAOCS 54:245A (1977).
- 32. Hagenmaier, R.D., Ibid. 51:470 (1974).
- Chen, Shiow-Ling, Thesis, Texas A&M University, College Station, TX, August 1976.
- Lawhon, J.T., S.H.C. Lin, D.W. Hensley, C.M. Cater and K.F. Mattil, J. Food Proc. Eng. 1:15 (1977).
- 35. Lawhon, J.T., S.H.C. Lin, C.M. Cater and K.F. Mattil, Food Technol. 27:26 (1973).
- Lawhon, J.T., D.W. Hensley, D. Mulsow and K.F. Mattil, J. Food Sci. 43:361 (1978).
- 37. Manak, L.J., J.T. Lawhon and E.W. Lusas, Ibid. 45:236 (1980).
- 38. Lawhon, J.T., N.H. Golightly and E.W. Lusas, JAOCS, in press.
- 39. Khan, M.N., and J.T. Lawhon, Cereal Chem., in press.
- 40. Hensley, D.W., and J.T. Lawhon, Food Technol. 33:46 (1979).