Functionality of Soy Protein Produced by Enzyme-Assisted Extraction

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ABSTRACT: This study investigated the potential of enzymes to increase soy protein extractability without causing protein degradation. The aqueous extraction of protein was performed from defatted soy flakes on a laboratory- and pilot-plant scale. Yields of protein and reducing sugars were determined in the alkali extracts obtained with cellulases and pectinase, added alone or as cocktails. Using 5% (wt/g of protein) Multifect pectinase resulted in the best improvement of protein yields, which were 50 and 17% greater than the controls in laboratory- and pilot-plant-scale trials, respectively. This enhanced protein extraction was accompanied by an increased reducing sugar content in the aqueous extract compared with the control. Under the conditions tested, no enzyme cocktail markedly increased the protein yield compared with the use of single enzymes. The solubility curve for Multifect pectinase-treated soy protein isolate (SPI) was typical of SPI at pH 2–10. Its foam stability significantly improved, but the emulsification properties declined. Multifect pectinase markedly reduced the viscosity of SPI. SDS-PAGE showed that the α′ and α subunits of β-conglycinin were modified, and glycoprotein staining showed that these modifications were probably due to a protease secondary activity in the pectinase preparation. One cellulase and one pectinase were identified as effective in modifying the protein and reducing sugar extractability from the defatted soy flakes.

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KEY WORDS: Cellulase, β-conglycinin, functional properties, glycinin, pectinase, protein extraction, soy protein.

Soy protein isolate (SPI) is one of three major types of soy protein ingredients commercially available, the two other products being soy protein concentrate and defatted soy flour. The process used for producing SPI, which contains at least 90% [dry basis (db)] crude protein, employs alkali to extract protein from defatted soy flour or flakes (1). The yield of protein extracted needs to be improved because only about one-half of the available protein is removed and the rest is largely lost in the spent flakes. Protein extractability depends on several parameters, including the severity of thermal treatment that the defatted soy flakes undergo during preparation (2). The mechanical process used to obtain the flour, for example, grinding or flaking, can also affect the protein extraction yield (3,4). This inefficient extraction represents a considerable loss of valuable protein. Indeed, the remaining protein-rich fiber fraction, a by-product of the oil and/or protein industry, has limited direct feed use because of several nutritional disadvantages, such as the presence of antinutritional factors (2), and therefore needs to be further processed to be useful.

Defatted soy flours and grits are composed of 56–59% protein, 32–34% carbohydrates, 5.4–6.5% ash, 2.7–3.8% crude fiber, and 0.5–1.1% free lipids (1). Fiber is not the major constituent of the defatted soy flour, but it plays a key role in protein extraction. Mudgett *et al.* (5) used a reinforced concrete analogy to describe the structural components of plant tissue: "The cellulose fibers of the cell wall are visualized as steel rods and the lamellar pectinic substances as concrete." Even though our understanding of plant cell walls has progressed, some knowledge gaps still need to be filled (6). Extraction of protein from this complex matrix involves several mechanisms, including dissolution and/or diffusion kinetics (4). Also, disrupting the integrity of the cell wall network might increase extractability of the major soybean components, i.e., oil and protein (7). This release might be obtained by using cell-wall-degrading enzymes, i.e., enzymes able to degrade celluloses, hemicelluloses, and/or pectin, which are the major components of plant cell walls and fiber (8,9).

Some research has focused on investigating the potential use of enzymes to enhance feed value, i.e., cell-wall-degrading enzymes acting on soybean cell walls to obtain products with enhanced nutritional value by breaking down the cell wall polysaccharides to smaller, more digestible saccharides (2,10). Other studies have examined the potential for carbohydrases to increase protein extractability, but only at a laboratory scale, using only a few grams of starting materials (4,7). The purpose of the present study was to determine whether carbohydrase preparations, as single enzymes or combinations of enzymes, could be used on a laboratory and pilot-plant scale to improve protein extraction yields from defatted soy flakes and to compare protein functionalities of a pectinase-modified SPI with corresponding controls.

MATERIALS AND METHODS

Soy flakes. Defatted (hexane-extracted) soy flakes for the laboratory experiments were purchased from the Archer Daniels Midland Co. (Decatur, IL). The flakes contained 8% moisture, 60.6% (db) crude protein, 0.34% (db) crude free lipid, and

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6.4% (db) ash. The protein dispersibility index (PDI), determined by Woodson-Tenent Labs (Des Moines, IA), was 84. Defatted soy flakes for pilot-plant trials were purchased from Cargill Inc. (Minneapolis, MN). These flakes contained 5% moisture and 53.5% (db) crude protein and had a PDI of 80.

Enzyme activities and characteristics. Four enzyme preparations having principally cellulase activity (E.C. 3.2.1.4.), known as IndiAge Super L, Puradax HA, Multifect B, and Multifect GC, and one preparation having pectinase activity (E.C. 3.2.1.15.), Multifect pectinase, were examined. According to the enzyme manufacturer, Multifect GC contained hemicellulase, xylanase, and glucanase activities. The activities of the enzymes were 3000 genencor textile units/g for IndiAge Super L, 410 *O*-nitrophenyl β-D-cellobioside units/g for Puradax HA, 2522 B-glucanase activity unit/g for Multifect B, 100 genencor cellulase unit/g Multifect GC and 8100 pectolytic activity unit/g for Multifect Pectinase. All enzyme preparations were provided by Genencor International (Rochester, NY). The temperatures (°C) and pH used were 45 and 7.0 for IndiAge Super L, 60 and 7.0 for Puradax HA, 50 and 5.0 for Multifect B, 55 and 4.0 for Multifect GC, and 50 and 4.0 for Multifect Pectinase, respectively. These conditions corresponded to the optimal conditions of each enzyme. The quantity of enzyme was determined as the weight of enzyme preparation (as is) per unit weight of soy flake protein (db).

Laboratory-scale extraction procedures. Extractions were performed with 30 g of defatted soy flakes and 1:6 flake-to-water ratio. The water was heated to the optimal temperature of each enzyme before adding to the flakes. Preliminary results showed that variation in the extraction temperature between 45 and 60°C did not affect protein and reducing sugar yields for the controls. The suspensions were adjusted to the desired pH, depending on the pH optima of the enzymes used, with 2 N NaOH or HCl. The slurry was stirred in a 1-L beaker. After completing the desired reaction time for extractions at pH >6.4, the slurry was centrifuged at 14,000 × *g* for 20 min at room temperature (RT). For extractions at pH <6.4, a 30-min extraction step at pH 7 was added before centrifugation.

Pilot-scale SPI preparation. Enzyme-modified SPI were prepared at pilot-plant scale from 15 kg of soy flakes and 1:15 flake-to-water ratio. The slurry was brought up to the extraction temperature of 60°C for the Multifect pectinase treatment and 50°C for the IndiAge Super L treatment in a jacketed 400-L tank (Walker Stainless Equipment Co., New Lisbon, WI) while being stirred at 22 rpm with a propeller stirrer. Some of the flakes clumped at the beginning and were broken up with a handheld paddle. The slurry was completely dispersed within 5 to 7 min. The pH was then adjusted to 4.0 for the Multifect pectinase treatment and to 7.0 for the IndiAge Super L treatment with 2 N HCl. The enzymes were then added at 5% (w/w db) enzyme-to-protein ratio based on laboratory-scale experiments, and extraction was carried out for 3 h while stirring at 13 rpm. Foaming was not a problem at this low stirring speed. The slurry was then adjusted to pH 8.5 with 2 N NaOH and to 60°C, and extracted for 30 min. This was the first step of the procedure used to obtain SPI. The slurry was then fed at *ca*. 2 L/min with a Moyno transfer pump to a continuous BTPX disc-stack centrifuge (Alfa Laval Separation Inc., Warminster, PA) set at 9,800 rpm bowl speed (*ca*. 12,000 × *g*). After sampling, the supernatant, which constituted an intermediate liquid fraction, was adjusted to pH 4.5 with 2 N HCl and kept at 20°C for 2 min before centrifuging at 9,800 rpm bowl speed. The resulting supernatant constituted the whey fraction. The protein curd was diluted with water and adjusted to pH 7 before spray drying in an Anhydro compact dryer (APV Crepaco Inc., Attleboro Falls, MA). From this procedure, three fractions were obtained: the insoluble spent flour fraction, the whey fraction, and SPI. The insoluble fraction and whey were weighed, sampled, and discarded. Protein and mass balances were determined. Two controls (no enzyme addition) were prepared, pH 4-control and pH 7-control, with solubilization steps at pH 4 or 7, respectively. The treatments in the pilot plant were duplicated.

Rheological flow behavior. Dispersions of the spray-dried SPI at 10% protein concentration (g protein/g water) and pH 7 were prepared. The rheological behavior of each sample was measured at 23°C by using an RS-150 Rheo Stress rheometer (Haake, Karlsruhe, Germany). Shear was applied by using a 60-mm 2° titanium cone over the range 10–500/s. The experimental flow curves were modeled by using the power law model: $\tau = K\gamma^n$, where τ was shear stress (Pa), K was the viscosity coefficient, γ was shear rate (s^{-1}), and n was the flow index. Samples were tested a minimum of three times.

Protein functionality. Protein solubility over the range of pH 2 to 10, emulsification properties (capacity, activity, and stability), and foaming properties (capacity, specific rate constant of drainage, and rate of liquid incorporation) were determined and compared using methods already described (11). SDS-PAGE was carried out to follow changes in the peptide M.W. as described by Jung *et al.* (12).

Glycosylation characterization. Glycoproteins were visualized by the periodic acid-Schiff (PAS) method and simultaneously stained with Coomassie blue for comparison (13). Ten to 15 μg of protein was loaded per well for PAS staining, whereas only 4 μg was loaded for Coomassie staining on urea-SDS-mini gels (Mini Protean 3 Cell; Bio-Rad Laboratories, Inc., Hercules, CA). Gels were run at 100 mV for 1 h. For PAS staining, the proteins were fixed in 12.5% trichloroacetic acid for 30 min. Gels were rinsed for 20 s in water and immersed for 50 min in 1% (wt/vol) periodic acid in 3% (vol/vol) acetic acid. Gels were washed overnight with 6 × 200 mL distilled water per gel. The gels were then immersed for 20 min in the dark in fuchsin-sulfite stain (S5133; Sigma, St. Louis, MO) and destained three times for 10 min with 0.5% (wt/vol) metabisulfite. Finally, distilled water was used to remove excess stain and the gels were stored in 7.5% acetic acid. For staining with Coomassie blue, the procedure described by Jung *et al.* (12) was used. Enzymatic deglycosylation of purified β-conglycinin was performed with N-Glycanase®PNGase F (Glyco®; Prozyme, San Leandro, CA) according to the manufacturer's instructions.

Analytical methods. The crude protein contents of solid and liquid samples were determined by using the Dumas method.

The basis of the Dumas method is the conversion of all nitrogen forms in the sample to nitrogen oxides through combustion, reduction of these forms to nitrogen gas, and subsequent measurement by use of a thermal conductivity detector (14). The total solids content was determined by drying samples at 130°C for 3 h (15). Carbohydrase activities were followed by estimating the rate of liberation of reducing sugar. The reducing sugar content in the extract was determined by using the Miller method with glucose as standard (16). Samples were diluted with water and boiled for 15 min in Eppendorf tubes containing 3,5-dinitrosalicylic acid solution. The samples were cooled in an ice-water bath and centrifuged at 14,000 × *g* for 2 min at RT. Absorbance was determined spectrophotometrically at 570 nm. Fiber analysis included neutral detergent fiber, acid detergent fiber, and acid detergent lignin was performed to determine the cellulose and hemicellulose composition of the starting material (17). Pectin content, calculated from the galacturonic acid and total neutral sugar contents, was determined by INRA (URPOI, Nantes, France) as described by Levigne *et al.* (18). Carbohydrate profiles of the SPI and defatted soy flakes were determined by NP Analytical Laboratories (St. Louis, MO). The samples (2 g) were extracted with 50 mL of a 1:1 mixture of denatured alcohol and water, passed through a 0.45-µm filter, and 20 µL was then analyzed by HPLC using a Waters 2410 refractive index detector.

Statistical analysis. All analyses and treatments were randomized and carried out in triplicate. SAS software (version 9.1.2, 2004; SAS Institute Inc., Cary, NC) was used for statistical analyses. Student's *t*-tests were performed to compare means. Statistical significance was determined at the $P < 0.05$ level.

RESULTS AND DISCUSSION

The primary objective of this study was to determine whether cellulase, hemicellulase, and pectinase preparations, which represented three groups of enzymes that may be suitable for the controlled breakdown of plant tissue structures (19), would increase protein extraction from defatted soy flakes in the laboratory, and to verify whether enhanced protein extractability can be scaled up to pilot-plant levels. The contents of cellulose, hemicellulose, and pectin in soybean products reported in the literature cover a broad range of values (1,4). Our starting material contained 4.5% cellulose, 0.8% hemicellulose, and 2.7% of pectin, as is. Cellulose appears to be a key component of the primary cell wall structure of soybeans, whereas pectin seems to be more involved in the secondary cell wall structure and acts as an adhesive between cells. However, the exact role of each of these constituents in the cell wall structural network and conditions that lead to increased protein extraction needs to be clarified.

Enzyme extractability in the laboratory. A 3-h extraction of defatted soy flakes increased the yield of protein by 9% using 1–10% of the cellulase Puradax HA and by 17% with 5 and 10% of the cellulase IndiAge Super L compared with the pH 7-control ($P < 0.05$, Table 1). This increase in protein yield was accompanied by an increase in reducing sugar concentration of approximately 6 units. Extraction time is an important process parameter because it may influence the extraction protein yield, i.e., the longer the enzyme can react with the substrate, the greater the hydrolysis of cell wall components. A longer hydrolysis time, however, will also increase processing costs and risk of microbial growth. Reducing the extraction time with 5% IndiAge Super L to 1 h gave extraction results similar to the control, while increasing it to 6 h gave results that were the same as the 3-h run (results not shown). This observation is consistent with that of Rosenthal *et al.* (7), who used response surface methodology and found that time alone (between 30 to 120 min), did not significantly affect protein and oil extractions from non-heat-treated soy flour treated with a cellulase from *Aspergillus niger* in laboratory experiments (500 mL). Marsman *et al.* (2), however, reported gradually enhanced protein extractability at 0.25, 1, 4, and 24 h of extraction with Protease Neutrase and cellulase Energex, added alone or in combination, using toasted, untoasted, and extruded soybean meals. It must be emphasized that the cellulase mixture Energex also contained protease activity. Using Energex cellulase on untoasted soybean meal (which was comparable to our starting material) increased protein extraction by approximately 8% after 4 h.

TABLE 1
Protein Extraction Yields and Reducing Sugar Contents of Defatted Soy Flour Extracts Obtained at Laboratory Scale with Single Enzymes[a]

Reaction at pH >6.4				Reaction at pH <6.4			
Enzyme	Enzyme concentration (%)	Protein extraction yield (%)	Reducing sugars (mg/g dry flake)	Enzyme	Enzyme concentration (%)	Protein extraction yield (%)	Reducing sugars (mg/g dry flake)
Puradax HA (pH 7.0)[b]	0	55.5[a]	27[a]	Multifect GC (pH 4.0)[b]	0	31.4[a]	13[a]
	1	60.6[b]	29[a,b]		10	35.5[b]	20[b]
	2	ND	30[a,b]				
	5	59.2[b]	31[a,b]	Multifect B (pH 5.0)[b]	0	46.0[a]	20[a]
	10	60.3[b]	34[b]		5	48.2[a]	24[a]
IndiAge Super L (pH 7.0)[b]	0	53.3[a]	27[a]	Multifect pectinase (pH 4.0)[b]	0	31.8[a]	11[a]
	1	58.2[b]	28[a]		1	38.5[a]	79[b]
	5	62.5[b]	28[a]		5	46.6[b]	110[c]
	10	62.4[b]	32[b]		10	48.2[b]	108[c]

[a]ND, not determined. For each enzyme, means in the same column followed by the same roman superscripts are not statistically different at $P < 0.05$.
[b]pH of the reaction. The reaction time was 3 h.

Incubation of the defatted soy flakes with cellulase Multifect GC, Multifect pectinase, and cellulase Multifect B was carried out at an extraction pH <6.4 due to the pH optima of these enzyme preparations. The addition of 5% Multifect B did not modify the protein extraction yield, whereas adding 10% Multifect GC gave a 13% higher protein extraction yield over the pH 4-control (Table 1). For extractions conducted with Multifect GC, the reducing sugar content of the supernatant increased from 13% for the pH 4-control to 20%, demonstrating that the cellulase was active. A 1% Multifect pectinase treatment did not significantly affect the protein extraction yield. Increasing the enzyme concentration to 5 or 10% increased the protein extraction yield by about 50% over the pH 4-control. The amount of reducing sugar increased almost 10-fold compared with the control (Table 1). It has been reported that treatment of soy pectin-rich fractions with a pectinase preparation increased the uronic acid as well as the arabinose, glucose, and galactose contents of the extract (20), suggesting side activities, such as endoarabinase, glucosidase, and galactosidase, in the enzyme preparation. Our pectinase preparation may also have contained some side activity contributing to the increased reducing sugar content. It would have been interesting to determine the carbohydrate profile of the alkali extracts to identify the potential of these carbohydrase preparations to obtain improved feed, i.e., improved digestibility. Indeed, the breakdown of cell wall constituents (i.e., cellulose, hemicellulose, and pectins) to small oligomers and monomers may be a means to increase the metabolizable energy in feed (2).

When the enzyme-assisted reaction was run at pH <6.4, the protein extraction yield for the control was lower than the one obtained at pH 7. Both cellulase Multifect GC and Multifect pectinase controls, which were prepared by conducting the first step at pH 4, achieved protein yields of around 30%, about one-half the protein extraction yield of the control conducted at pH 7. With Multifect B, extraction was carried out at pH 5 and a higher yield was obtained compared with the controls at pH 4 (46 vs. *ca*. 32%). These low protein yields were attributed to the pH used for extraction (pH 4–5), which coincided with the minimum solubilities of glycinin and β-conglycinin (1), and confirmed the results obtained in previous studies (2,5). Our results revealed that pH adjustment to a value of high protein solubility (pH 7), followed by stirring for 30 min, was insufficient to totally resolubilize the precipitated proteins.

Protein extraction yields of up to 29.3% were reported by Marsman *et al.* (2) when untreated and toasted soybean meals were treated with 0.25% of various cellulases. Our lower extraction yield of 17% with cellulase IndiAge Super L may have been due to the lower extraction reaction time, which was 3 h vs. 24 h in the study by Marsman *et al.* Studies reporting the use of enzymes to improve oil or protein extraction from soybean meal have usually been conducted with enzymes that are not pure because of the high cost of using high-purity enzymes. These enzyme preparations contain secondary activities. This might be one reason for differences between studies regarding enzyme efficiency, in addition to differences in the substrate characteristics and experimental conditions used. In our study when a single cellulase was evaluated, the protein extraction yield gradually improved in the following order: Multifect GC, Multifect B, Puradax HA, and finally IndiAge Super L (protein extraction yield increased 17% over the control). The most efficient enzyme was Multifect pectinase, which improved the protein extraction yield by 50% over the pH 4-control; thus, in the experimental conditions tested, pectin seemed to play a key role in protein release compared with cellulose and hemicellulose. Pectic materials, which are the principal constituents of the middle lamellar structure and provide adhesion between cells, were also identified by Kasai *et al.* (19) as important structural components of the secondary cell wall. They showed that cellulase treatment (40°C for 15 h) of autoclaved okara, i.e., soybean fiber residue of soymilk production, was effective in digesting the primary cell wall, whereas pectin treatment was effective in digesting the secondary cell wall. It would have been interesting to investigate the effects of other pectinases or pectinolytic enzymes such as polygalacturonases, pectate lyases, or pectin methyl esterases to determine their potential in increasing protein extractability without causing protein degradation.

Combinations of enzymes. The impacts of carbohydrase cocktails were investigated to identify synergistic effects of carbohydrases on protein extractability; in particular, a cocktail of cellulase and pectinase was investigated as a potential tool to alter the primary and secondary walls. When IndiAge Super L and Puradax HA at 5% concentration each (wt enzyme/wt protein) were added to the soy flakes and incubated at 50°C and pH 7 for 3 h, this enzyme cocktail increased protein extraction by 12% and increased reducing sugar production by more than 50% compared with the control (Table 2). The increased protein extraction was

TABLE 2
Protein Extraction Yields and Reducing Sugar Contents of Defatted Soy Flake Supernatants Obtained at Laboratory Scale with Enzyme Combinations[a]

	Simultaneous addition of enzymes[b]		Consecutive addition of enzymes[c]	
	Protein extraction yield (%)	Reducing sugars (mg/g dry flake)	Protein extraction yield (%)	Reducing sugars (mg/g dry flake)
Control	56.9[a]	27[a]	64.7[a]	31[a]
Enzyme-treated	63.2[b]	43[b]	63.3[a]	33[a]

[a]Means in the same column followed by the same roman superscript are not significantly different ($P < 0.05$).
[b]Cocktail of Puradax HA and IndiAge Super L (5% each). Reaction conditions: pH 7.0, 50°C, 3 h.
[c]After 1 h of reaction with 5% cellulase IndiAge Super L, the slurry was centrifuged and the precipitate was resuspended in 180 mL of 60°C distilled water. The pH was then adjusted to 4, and 10% Multifect GC was added to the extract. After 2 h the pH was adjusted to 7 and the slurry was stirred for 30 min.

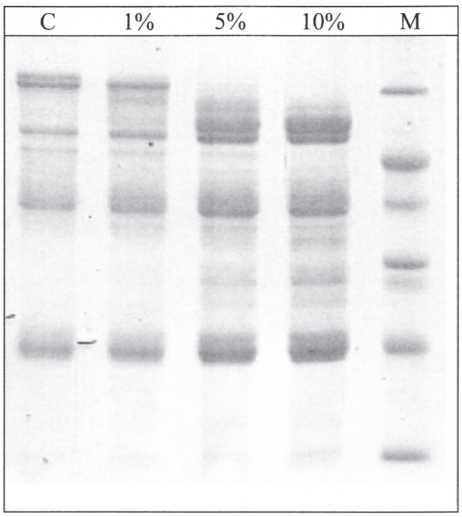

FIG. 1. SDS-PAGE profiles of the protein extracts obtained with 1, 5, and 10% of Multifect pectinase: C, control; M, M.W. marker (66, 45, 36, 29, 24, 20, and 14 kDa).

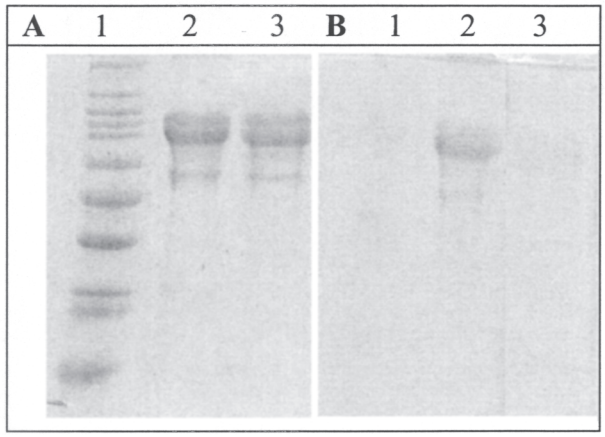

FIG. 2. Urea-SDS-PAGE of soy protein isolates after (A) Coomassie blue staining and (B) periodic acid-Schiff (PAS) staining. (1) SigmaMarker™ Wide (205, 116, 97, 84, 66, 55, 45, 36, 29, 24, 20, 14.2, 6.5 kDa); (2) purified β-conglycinin; (3) purified β-conglycinin treated with PNGase F (removal of the N-linked oligosaccharides).

in the same range as for 5% IndiAge Super L alone, indicating there was no synergistic effect on protein extraction when these two enzymes were added together.

Combinations of cellulases added consecutively were also investigated. A 1-h reaction with 5% IndiAge Super L did not modify the protein extraction yield compared with the corresponding control, and further addition of Multifect GC did not improve the final protein extraction yield (Table 2). The consecutive addition of 5% IndiAge Super L to a slurry first extracted with 10% Multifect pectinase for 3 h at pH 4 did not enhance the protein extraction compared with extraction without IndiAge Super L. The protein extraction yield and reducing sugar production were the same as those achieved with Multifect pectinase alone (results not shown). The synergistic effect of the cellulase–pectinase mixture on protein extraction obtained with alfalfa leaves was not observed in our experimental conditions with soybeans (5). Even if some of the enzyme cocktails increased the reducing sugar yield, none of them significantly increased protein extraction compared with single enzymes. Therefore, no synergistic effect between enzymes was identified for improved protein extraction.

Peptide profile. The peptide profile of the extracts obtained with cellulases IndiAge Super L, Puradax, Multifect B, and Multifect GC treatments were similar to a traditional soy protein water extract (results not shown), indicating that no side protease activities were present in any of the enzyme preparations or that the protease did not modify the peptide profiles in the conditions tested. The SDS-PAGE profile for soy proteins after treatment with pectinase showed the disappearance of the α, α′, and β subunits of the β-conglycinin using 5 and 10% pectinase, and the appearance of new peptides (Fig. 1). Similar degradation patterns were observed when the Multifect pectinase/cellulase IndiAge Super L mixture was used. The apparent M.W. of the new peptides were around 58, 53, 33, and 26 kDa.

β-Conglycinin is a glycoprotein made up of three subunits of 57 kDa for α and α′, and 42 kDa for β, as determined by gel filtration (21). The presence of water associated with the oligosaccharide chains increased the apparent molecular size of α, α′, and β subunits when observed by SDS-PAGE (22). Consequently, deglycosylation of this glycoprotein should result in a shift of the M.W. of its subunits. To determine whether the 58-kD polypeptide that appeared after Multifect pectinase treatment corresponded to deglycosylated α and α′ subunits, PAS staining was used to reveal the presence of carbohydrates associated with β-conglycinin subunits. When purified β-conglycinin was treated with pure PNGaseF, known to remove the N-linked oligosaccharides of glycoprotein (23), and further stained with PAS, no staining of the subunits was observed, thus confirming the removal of carbohydrate moieties (Fig. 2). This pattern was not observed with the Multifect pectinase-treated samples, revealing the presence of a high amount of sugar linked to the 58-kDa polypeptide (Fig. 3). This polypeptide and other lower-M.W. peptides apparently derived from β-conglycinin subunits could therefore not be the result of deglycosylating α and α′ subunits, and their appearance was probably due to protease acting as a side activity in the Multifect pectinase preparation.

Pilot-plant extraction and isolate characterization. Two enzymes were selected to prepare SPI at the pilot-plant scale: Multifect pectinase and IndiAge Super L cellulase. Protein extraction yields for the controls prepared with the first steps at pH 7 and pH 4 were 57 and 46%, respectively (Table 3). These values confirmed the laboratory results regarding the impact of pH on protein extraction yield. After the acidifying, centrifuging and spray-drying steps, SPI yields were 28 and 41% for the pH 4- and pH 7-controls, respectively. Wu *et al.* (24) reported a 41.5% protein yield during SPI production. Multifect pectinase gave the best protein extraction yield, increasing the yield by 17% over the pH 4-control, while the cellulase gave only a 4% increase.

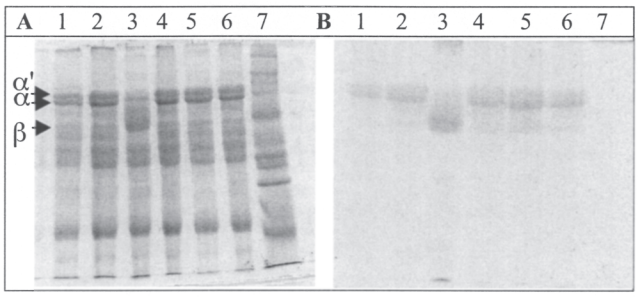

FIG. 3. Urea-SDS-PAGE of soy protein isolates after (A) Coomassie blue staining and (B) PAS staining. (1) Defatted soy flake extract; (2) pH 7-control; (3) Multifect-pectinase-treated extract; (4) IndiAge Super L-treated extract; (5) pH 4-control; (6) pH 7-control; (7) SigmaMarker™ Wide Range (205, 116, 97, 84, 66, 55, 45, 36, 29, 24, 20, 14.2, 6.5 kDa). Arrows indicate the location of the β-conglycinin subunits. For abbreviation see Figure 2.

Both values were lower than those obtained in the laboratory. However, this increased protein extraction over the control was not maintained throughout the process. A significant amount of protein remained in the whey fraction after extraction with Multifect pectinase; thus, the final SPI protein yield of the pectinase-treated sample was similar to its corresponding pH 4-control. The reducing sugar content of the Multifect pectinase extract increased by a factor of 3.5, whereas a factor of 10 was obtained in the laboratory. As observed at laboratory scale, the peptide profile of the SPI was modified with the use of Multifect pectinase, whereas no change was apparent with IndiAge Super L. It is well known that modifications of the peptide profile indicate altered protein functionality (12). Consequently, the functionality of Multifect pectinase-treated SPI was determined and compared with the pH 4- and pH 7-control SPI.

Characterization of pilot-plant-made SPI. Solubility profiles of the control SPI were typical U-shaped curves, with the lowest solubility around the protein isoelectric point (pH 4–5). No significant changes in solubility behavior were observed for the enzyme-treated samples (results not shown). Lowering the dispersion pH value (4 vs. 7) significantly reduced SPI emulsifying properties. Pectinase treatment further decreased the emulsifying properties (Table 4). The foaming capacities of the Multifect pectinase-treated and control SPI were similar. Improved foam stability was obtained for the pectinase-treated SPI, as illustrated by lower specific drainage rate constant (*K*) values compared with the controls (Table 4).

The SPI obtained with Multifect pectinase had significantly lower shear stress values compared with both the pH 4- and pH 7-controls (Fig. 4). The *n* values were 0.96, 0.85, and 0.83, for the enzyme-treated, pH 4-, and pH 7-samples, respectively. The pectinase-treated SPI tended to behave as a Newtonian fluid (i.e., with the *n* value moving closer to 1). This modification of the viscosity behavior cannot be attributed to improved protein solubility, as no change was observed after pectinase treatment, but it may be related to a protein conformational change and/or the production of new peptides, as observed by SDS-PAGE.

The carbohydrate profiles of the enzyme-treated SPI were analyzed to determine whether there were changes in the raffinose and stachyose contents, carbohydrates that can cause flatulence due to bacterial fermentation in the colon, creating intestinal gas (25). The defatted soy flakes contained mainly stachyose and disaccharides, followed by raffinose, glucose, galactinol, and fructose (Table 5). These results were similar to those of Huisman *et al.* (26), who reported 4.9% stachyose and 5.4% sucrose in untoasted soybean meal. The total carbohydrate contents of SPI were not significantly affected by enzyme treatment and were reduced by a factor of 4 compared with the starting defatted soy flakes (11.13 vs. average 2.79%). Because of the low residual carbohydrate content of SPI and the variability between the pectinase-treated replicates, there were no statistical differences between the treated and untreated samples, except in fructose content. Treatment with IndiAge Super L did not modify the carbohydrate profile of SPI compared with the control.

In the experimental conditions tested, a modest increase in the protein extraction yield was obtained by using carbohydrases at laboratory scale; however, this yield was less when scaled up. Carbohydrases have been reported to have only a limited effect on intact soybean cell walls (20), and treating defatted soybean flakes with our cellulase preparations led us to the same conclusion. We identified a pectinase mixture that significantly increased the protein extraction yield compared with the pH 4-control, and this preparation may be useful for SPI production since its use resulted in a product with improved foaming stability and lower viscosity. The use of drastic thermal treatment (i.e., autoclaving, in combination with carbohydrase treatment) was recently reported as an efficient procedure to improve protein extractability from the soybean fiber residue of soymilk production (okara). However, a thermal treatment at 121°C for 20 min may dramatically alter the functionality of the extracted proteins. Maintaining protein functionality by

TABLE 3
Crude Protein and Reducing Sugar Contents of Fractions Obtained Through Soy Protein Isolate Production with Enzymes at Pilot-Plant Scale[a]

	Extract			Protein isolate		Whey	
Treatment	Protein extraction yield (%)	Reducing sugars (mg/g dry flake)	Crude protein content of fiber fraction (%)	Crude protein content (%)	Protein extraction yield (%)	Crude Protein content (%)	Reducing sugars (mg/g dry flake)
pH 4-control	46.4^{c}	27^{b}	46.2^{a}	91.4^{a}	28.1^{b}	14.7^{a}	22^{a}
pH 7-control	57.3^{b,a}	41^{b}	36.7^{b,c}	91.9^{a}	41.5^{a}	12.2^{a}	27^{a}
Multifect pectinase	54.3^{b}	90^{a}	39.4^{b}	89.0^{a}	28.0^{b}	23.1^{b}	79^{b}
IndiAge Super L	59.8^{a}	41^{b}	34.8^{c}	92.8^{a}	40.7^{a}	13.0^{a}	31^{a}

[a]Means in the same column followed by the same roman superscript are not significantly different ($P < 0.05$).

TABLE 4
Emulsifying and Foaming Properties of Multifect Pectinase-Treated Soy Protein Isolate[a]

	Emulsification properties			Foaming properties		
Treatment	Emulsification capacity (g oil/g protein)	Emulsion activity (min)	Emulsion stability (min)	Foaming capacity (units)	K (1/mL · min × 10^{-3})[b]	V_i (mL/min)[b]
pH 4-control	562[a]	0.308[a]	270[a]	1.8[a]	10.5[b]	36.6[b]
pH 7-control	755[b]	0.364[b]	341[b]	1.8[a]	20.7[a]	28.0[a]
Multifect pectinase	465[c]	0.226[c]	132[c]	1.7[a]	7.4[c]	35.7[a,b]

[a]Means in the same column followed by same superscript are not significantly different ($P < 0.05$).
[b]K and V_i correspond to the drainage constant and rate of liquid incorporation, respectively.

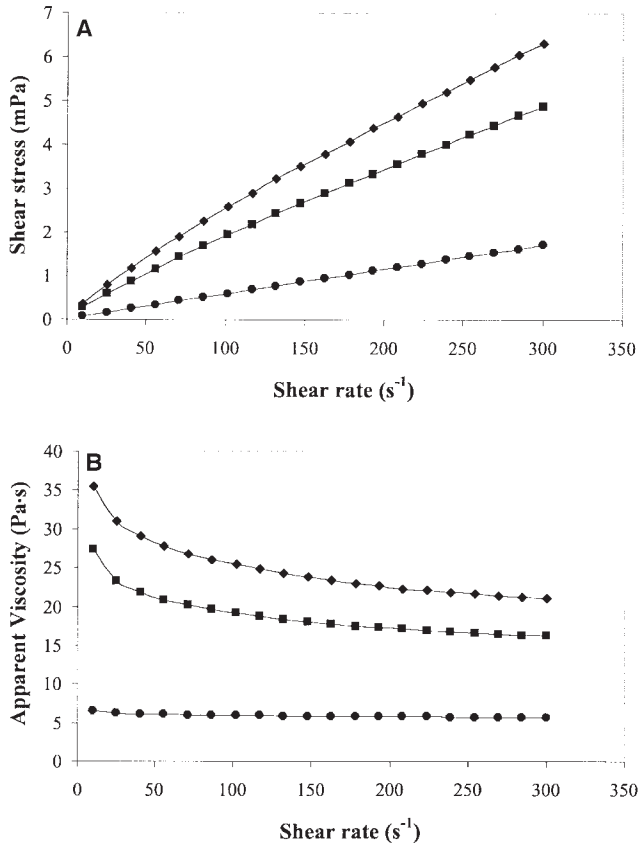

FIG. 4. Shear stress (A) and apparent viscosity (B) vs. shear rate for the pH 7-control (◆), pH 4-control (■), and Multifect-pectinase-treated samples (●).

keeping the original structural conformations was a major concern in our study and explains why no protease treatment was performed. Our work revealed that protease secondary activity might, however, be present in carbohydrase preparations and that it alters the functional properties of extracted proteins. More investigations are currently underway to characterize structural modifications to the fiber fraction attributable to enzymatic treatment and the potential of a physical processing step before or after the enzyme treatment to help the enzyme access its substrates and release protein. It would also be interesting to determine the fate of isoflavones during enzyme-assisted soy protein extraction technology, as these phytochemicals add value to soy products, and about 30% of the total isoflavones are lost in the insoluble fraction during SPI production (27). Based on the available literature, aqueous extraction of protein and isoflavones seems to be influenced by similar parameters; therefore, we hypothesized that an increase in protein extraction would improve isoflavone extractability.

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TABLE 5
Carbohydrate Contents (%) of Defatted Soy Flakes and Enzyme-Treated Soy Protein Isolates and Their Corresponding Controls[a]

	Sugar profile						
Treatment	Stachyose	Raffinose	Disaccharides[b]	Galactinol	Glucose	Fructose	Total
Defatted soy flakes	4.75 ± 0.42	0.92 ± 0.08	4.84 ± 0.38	0.14 ± 0.01	0.43 ± 0.03	0.06 ± 0.02	11.13 ± 0.95
				Group A			
pH 7-control	1.31 ± 0.15	0.21 ± 0.04	0.87 ± 0.09	0.02 ± 0.00	0.21 ± 0.05	0.13 ± 0.01	2.75 ± 0.35
IndiAge Super L	1.27 ± 0.14	0.22 ± 0.05	0.89 ± 0.17	0.01 ± 0.00	0.21 ± 0.05	0.16 ± 0.02	2.78 ± 0.33
				Group B			
pH 4-control	1.33 ± 0.52	0.25 ± 0.13	1.14 ± 0.49	0.02 ± 0.01	0.15 ± 0.08	0.07 ± 0.03	3.01 ± 1.29
Multifect pectinase	1.16 ± 0.08	0.30 ± 0.04	0.89 ± 0.17	0.01 ± 0.00	0.24 ± 0.08	0.23* ± 0.06	2.64 ± 0.37

[a]Means ± SD; means followed by an asterisk in the same group are significantly different from the corresponding controls.
[b]Disaccharides included sucrose, maltose, and lactose.

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